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Supercritical CO_2 Extraction of Carotenoids from Carrots and Characterization of Products

by ©

Mei Sun

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in

Food Science and Technology

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ABSTRACT

Carotenoids and dietary fibre are nutraceutical components in carrot. Carotenoids can be extracted with supercritical CO_2 (SC-CO_2) without the use of organic solvents. Canola oil was investigated as a co-solvent in the SC-CO_2 extraction of carotenoids from carrots. Carrot samples with different particle size and moisture content were extracted at different temperatures, pressures, canola oil concentrations and CO_2 flow rates. Canola oil had a significant ($p<0.05$) positive effect on the carotenoid yields. The highest carotenoid yields were obtained at 70 °C, 55.1 MPa, 5% canola oil concentration, 0.25-0.5 mm particle size, 0.8% moisture content of feed material and 2 L/min CO_2 flow rate. The carotenoid crystals, carotenoid-saturated oil and carrot fibre all had high antioxidant activity compared to that of butylated hydroxytoluene. The fibre residue also had high water binding, oil binding and swelling capacity. Use of canola oil as a co-solvent shows great potential for the SC-CO_2 extraction of carotenoids.

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LIST OF ABBREVIATIONS

AA	Antioxidant activity
AMD	Age-related macular degeneration
BHT	Butylated hydroxytoluene
CEC	Cation-exchange capacity
CHD	Coronary heart disease
DF	Dietary fibre
DP	Degree of polymeration
FDA	Food and Drug Administration
FTC	Ferric thiocyanate
HPLC	High-performance liquid chromatography
IDF	Insoluble dietary fiber
LLL	Trilaurin
LLO	Olein-dilinolein
LnLO	Linonenin-linolein-olein
LnOO	Linolenin-diolein
LOO	Linolein-diolein
MES	Two-(N-morpholino)-ethanesulfonic acid
MMM	Trimyristin
NCP	Noncellulosic polysaccharides
NSP	Non-starch polysaccharides
OBC	Oil binding capacity
OI	Oxidation index
OOO	Triolein
P	Pressure
POO	Palmitin-diolein
PPO	Olein-dipalmitin
PPP	Tripalmitin
RS	Resistant starch
SC-CO_2	Supercritical carbon dioxide
SCF	Supercritical fluid
SDF	Soluble dietary fibre
SFE	Supercritical fluid extraction
T	Temperature
TDF	Total dietary fiber
TG	Triglycerides
TRIS	Tris (hydroxymethyl) aminomethane
TSE	Traditional solvent extraction
WBC	Water binding capacity

1. INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION AND OBJECTIVES

1.1.1. Introduction

Just as bacteria, viruses, fungi, and parasites can destroy our health, so can an abundance of free radicals. If the bacteria theory of disease dominated the medical theory of the 19th century, the free radical theory is the one dominating the 20th century. Free radicals are generated from both the normal oxidation metabolism in our body and oxidation caused by environmental factors. In the past 150 years, various chronic diseases have reached epidemic proportions due to an over abundance of free radicals. Various antioxidants have been evaluated as quenchers of free radicals. Correspondingly, functional foods and nutraceuticals have emerged as a new market on global scale. The term "functional food" was first introduced in Japan to describe food products offering certain health benefits. According to Health Canada, "a functional food is similar in appearance to, or may be a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions." In addition, "a nutraceutical is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease" (Anonymous, 2003a). Carotenoids, dietary fibre (DF), fatty acids, flavonoids, phenols, plant stanols/sterols, saponins, soy protein and prebiotic/probiotics are the main functional components (Anonymous, 2003b).

Carrots are a good source for both carotenoids and dietary fibre. Carotenoids are the main pigments in carrots, which contribute their orange color. In addition to their coloring effect, carotenoids also have pro-vitamin A and antioxidant activities. Acting as both a natural colorant and a natural antioxidant, carotenoids are experiencing a rapid growth in the market place, as they were shown to have a potential protective effect against some degenerative diseases (Mathews-Roth, 1985; Bendich and Olson, 1989; Ziegler, 1991; Krinsky, 1994). On the contrary, synthetic colorants and antioxidants are generally not as well accepted by consumers. The disease protective effects of carotenoids were mainly due to their antioxidant property through the deactivation of free radicals and singlet oxygen quenching (Burton, 1989; Krinsky, 1989).

Dietary fibre is a collection of substances of plant origin and escapes the breakdown by human digestive enzymes. High dietary fibre consumption has been associated with the prevention and treatment of some diseases such as coronary heart disease, diabetes, colon cancer, and diverticulitis (Mendeloff, 1987; Tinker et al., 1991; Anderson et al., 1994). The health effects of fibre are not only dependent on the quantity of fibre present in the diet but also on its composition. The insoluble fibre is related to water absorption and intestinal regulation, whereas the soluble fibre is associated with the reduction of cholesterol and glucose absorption. Over the past few years, a new concept of antioxidant dietary fibre has been introduced (Saura-Calixto, 1998). The antioxidant activities of plant dietary fibre were studied by Larrauri et al. (1996 and 1997), Saura-Calixto (1998), and Jimenez-Escrig et al. (2001) with pineapple core, red grape peel, and guava fruits, respectively.

In agriculture, the defect and culled fruits and vegetables cost the producers each year not only in terms of direct market loss but also an indirect disposal cost. In the food processing industry, similar problems are encountered by the food processors due to the processing waste. To minimize the economic loss of carrot producers and processors and to recover the nutraceutical components in the carrot waste, carotenoids can be extracted from these carrot waste by-products to produce a natural pigment of high nutritional value and the carrot extraction residue can be utilized as a dietary fibre supplement.

In the past few decades, carotenoids have been mainly extracted with organic solvents (O'Day and Rosenau, 1982; Aravantinos-Zafriris et al., 1992; Zelkha, et al., 1997; Todd, 1998). However, the uses of organic solvents are restricted by government regulations due to growing consumer concerns. On the other hand, supercritical CO_2 (SC-CO_2) extraction has been found to be an effective alternative. The extraction of carotenoids from carrots with SC-CO_2 alone or with SC-CO_2 plus a small amount of organic co-solvent has been studied (Goto et al., 1987; Favati et al., 1988; Barth et al, 1995; Sangbin and Mi, 1995; Vega et al., 1996; Ollanketo et al., 2001). However, the organic co-solvent added to the SC-CO_2 always casts a shade on this unique extraction technique, since the co-solvent has to be removed from the final product and the residue. Therefore, a co-solvent, which can improve the extraction efficiency of SC-CO_2 and itself is a natural edible material such as ethanol and vegetable oil, is needed. To date, there are no reports regarding the use of vegetable oil as a co-solvent for the extraction of carotenoids with SC-CO_2. Besides, the physicochemical properties of the residual fibre product, especially the antioxidant activity have not been evaluated. Therefore, this research was designed to fill the information gap in these areas.

1.1.2. Objectives

The overall objectives of this thesis research were to recover high-value components such as carotenoids using SC-CO_2 extraction with vegetable oil as a co-solvent. The specific objectives of the thesis were:

1. to investigate the use of vegetable oil as a co-solvent to extract carotenoids from carrot using SC-CO_2 (Chapter 2), and
2. to investigate the physical and chemical properties of the extract and residual carrot fibre after carotenoid extraction (Chapter 3).

1.2. CAROTENOIDS

A large number of pigments in living organisms, such as carotenoids, porphyrins, and flavonoids provide the rich variety of color in nature (Table 1.1). The carotenoids, believed to have derived their name from the fact that they constitute the major pigment in the carrot (*Daucus carota)* root, are among the most widespread and important pigments. This group of pigments is synthesized exclusively by photosynthetic microorganisms and by members of the plant kingdom where they play a fundamental role in metabolism. Although their presence is often masked by chlorophyll, carotenoids are found throughout the plant kingdom. They can also be found in algae, fungi, bacteria, insects, fish, birds and other animals. These pigments provide a whole range of light yellow to dark red colors, and when complexed with proteins, green and blue colorations are achieved (Tee, 1992). Certain food color extracts obtained from natural sources such as palm oil, carrot oil, paprika, annatto and saffron owe their color principally to

Table 1.1. Major classes of pigments and their colors[1]

Pigment class	Compound type	Color
Carotenoid	Carotenes (α, β and γ)	Yellow, orange and red
	Lycopene	Red
	Lutein	Yellow
	Canthaxanthin	Orange-red
	Apo-8'-carotenal	Yellow-red
	Astaxanthin	Red
Porphyrin	Chlorophyll	Green
Flavonoid	Flavone	Yellow
	Flavonol	Yellow
	Anthocyanin	Red, blue, purple and magenta

[1] Adopted from Anonymous (2003f)

carotenoids. It has been estimated that about 100 million tones of carotenoids are produced annually in nature (Gordon and Bauernfeind, 1982).

With the discovery of carotenoids and the many colors they provide, synthetic "natural identical" carotenoid colorants have been developed for food coloring. Along with β-carotene, the most prevalent synthetic colorant, lycopene, lutein, canthaxanthin, apo-8'-carotenal, and astaxanthin are commercially available in powder and liquid formulations for oil- or water-based food applications. Natural carotenoids are suggested to be safer and more effective than synthetic carotenoids. A study on smokers (The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, 1994) suggested that synthetic β-carotene supplementation may increase the risk of lung cancer. Synthetic β-carotene was shown to be not as effective as natural β-carotene in the reduction of oxidation in humans and it was not as well absorbed as natural mixed carotenoids (Omenn et al., 1996; Ben-Amotz and Levy, 1996)

1.2.1. Brief history of carotenoids

Researchers from diverse fields were fascinated by the brightly colored carotenoid pigments more than a century ago. Studies on these pigments have been traced to the beginning of the 19th century. Wachenroder (1831) named the yellow hydrocarbon pigment he isolated from carrot (*Daucus carota*) roots "*carotene*" and Berzelius (1837) named the more polar yellow pigments from autumn leaves "*xanthophylls*". These two terms, carotenes and xanthophylls, are widely accepted today. They represent the two groups of carotenoids with different characteristics: carotenes represent the hydrocarbon carotenoids such as lycopene and α-, β- and γ-carotenes and xanthophylls represent the oxygenated derivatives of carotenes such as lutein, zeaxanthin and canthaxanthin. In the early 20^{th} century, it was clearly shown that a whole family of carotenoids existed and Tswett (1911) called the whole family of pigments he chromatographically separated as "carotenoids". Crystalline β-carotene, prepared by chemical synthesis was first introduced commercially by Roche Vitamins and Fine Chemicals in 1954 (Gordon and Bauernfeind, 1982).

1.2.2. Structures of carotenoids

The basic structure of carotenoids is illustrated by the shorthand form of lycopene (Fig. 1.1), which consists of eight five-carbon isoprenoid units (*ip*). These eight isoprenoids are joined in the normal head-to-tail manner, except at the center of the molecule where the order is reversed so that the C_{40} skeleton is symmetrical when viewed as a whole. Thus, the two central methyl groups are in a 1, 6 position relative to each other while the remaining non-terminal methyl groups are in a 1, 5 relationship. Using the abbreviation of *ip* for the isoprenoid residue, the C_{40} carotenoids can be represented as: *ipipipip-pipipipi.*

Based on the structure of lycopene, other carotenoids can then be considered as related to it by means of structural changes in one or both halves of the molecule. These changes may be brought about by reactions involving: 1) hydrogenation, 2) dehydrogenation, 3) cyclization, 4) insertion of oxygen in various forms, 5) double bond migration, 6) methyl migration, 7) chain elongation, and 8) chain shortening. Though the number of basic structural modifications is comparatively few, their occurrence in different combinations accounts for the remarkable variety of natural carotenoids. More than 600 individual carotenoids have been isolated and identified in nature (Britton, 1995). The hydrocarbon carotenoids can be considered as a group of compounds, which

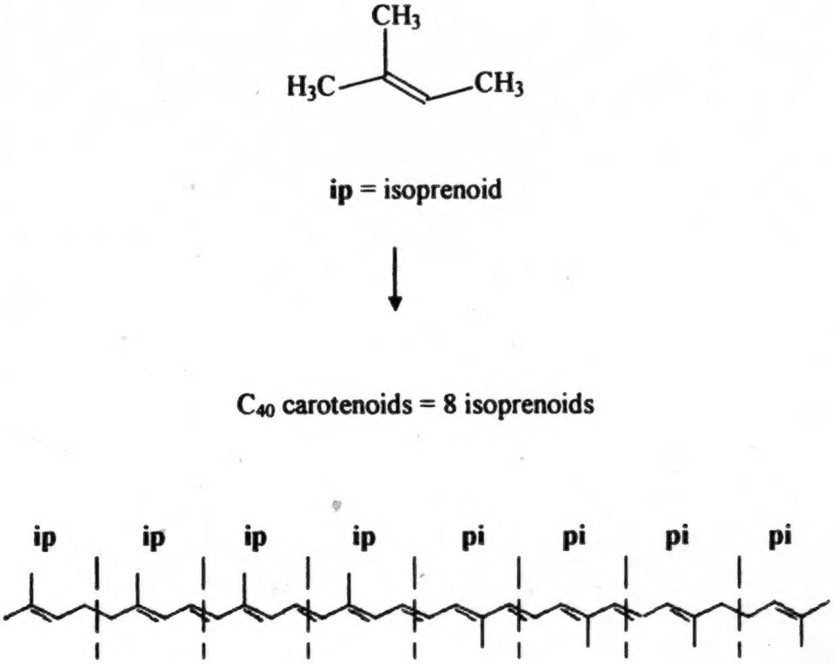

CH3
H3C
CH3
ip = isoprenoid
C40 carotenoids = 8 isoprenoids
ip
ip
ip
ip
pi
pi
pi
pi

Figure 1.1. The shorthand form of lycopene structure

contain different end groups that are generated from the double bond equivalents at two terminations (Fig. 1.2). The semi-circles in Figure 1.2 represent the double-bond equivalents. The seven C_9 end groups with a specific Greek letter and the stem were also shown in Figure 1.2. The oxygenated carotenoids have oxygen inserted at the terminations with functional groups such as hydroxyl, methoxyl, carboxyl, oxo and epoxy. The major carotenoids found in humans are β-carotene, lycopene, α-carotene, lutein, zeaxanthin and β-cryptoxanthin (Cooper, 1999). The shorthand forms of some other important carotenoids are listed in Figure 1.3.

1.2.3. Physical and chemical characteristics

The most striking and characteristic feature of the carotenoid structure is the long chain of alternating double and single bonds that forms the central part of the molecule (chromophore). The π-electrons are effectively delocalized over the entire length of the polyene chain. It is this feature that gives the carotenoids as a group their distinctive physicochemical properties, and hence, functions.

1.2.3.1 Physical characteristics

The carotenoids as a group are extremely hydrophobic molecules with little or no solubility in water. In aqueous media, they tend to form aggregates or adhere to surfaces. They are slightly soluble in vegetable oils, moderately soluble in aliphatic and aromatic hydrocarbons, and very soluble in chlorinated hydrocarbons (e.g. chloroform). The presence of chlorine in solvents appears to enhance the solubility of β-carotene, but the presence of fluorine or hydrogen bonding inhibits its solubility. The solubility of β-carotene in supercritical fluids and nearcritical fluids is generally much lower than its solubility in organic solvents (Lenfant and Thyrion, 1996).

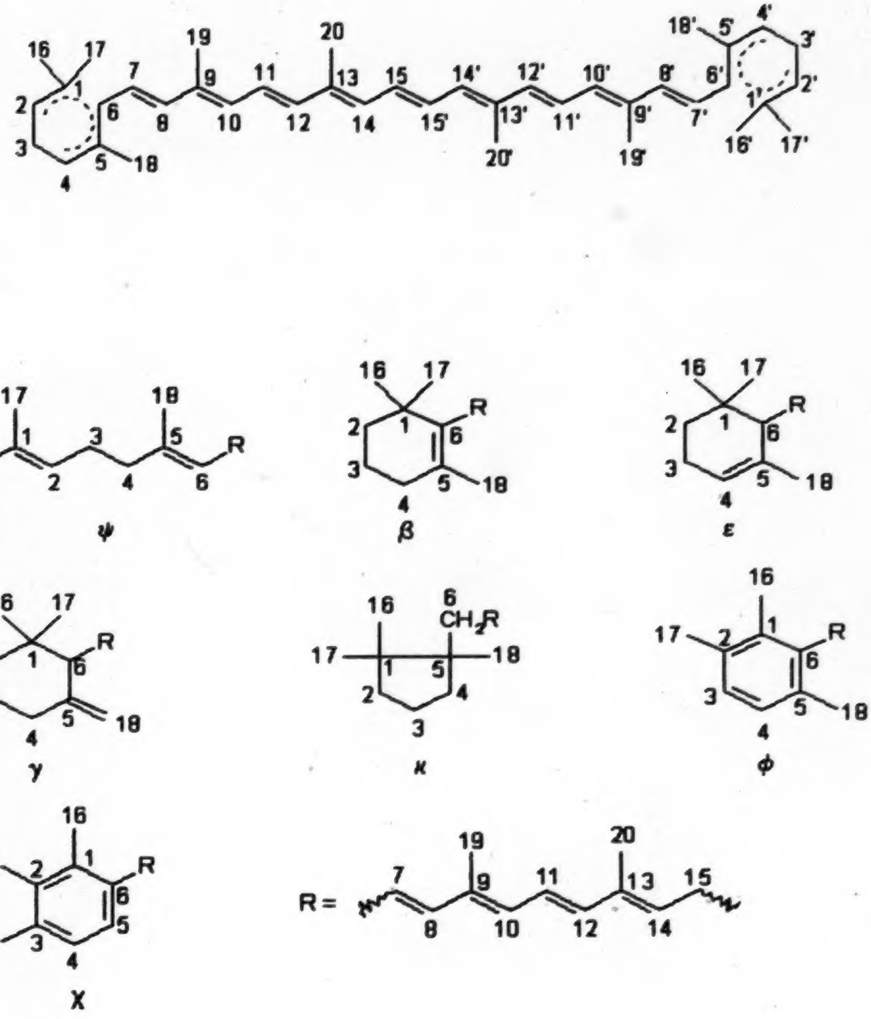

ψ
β
ε
γ
κ
ϕ
χ
R =
CH₂R

Figure 1.2. The structure and numbering of parent carotenoid, end groups and stem

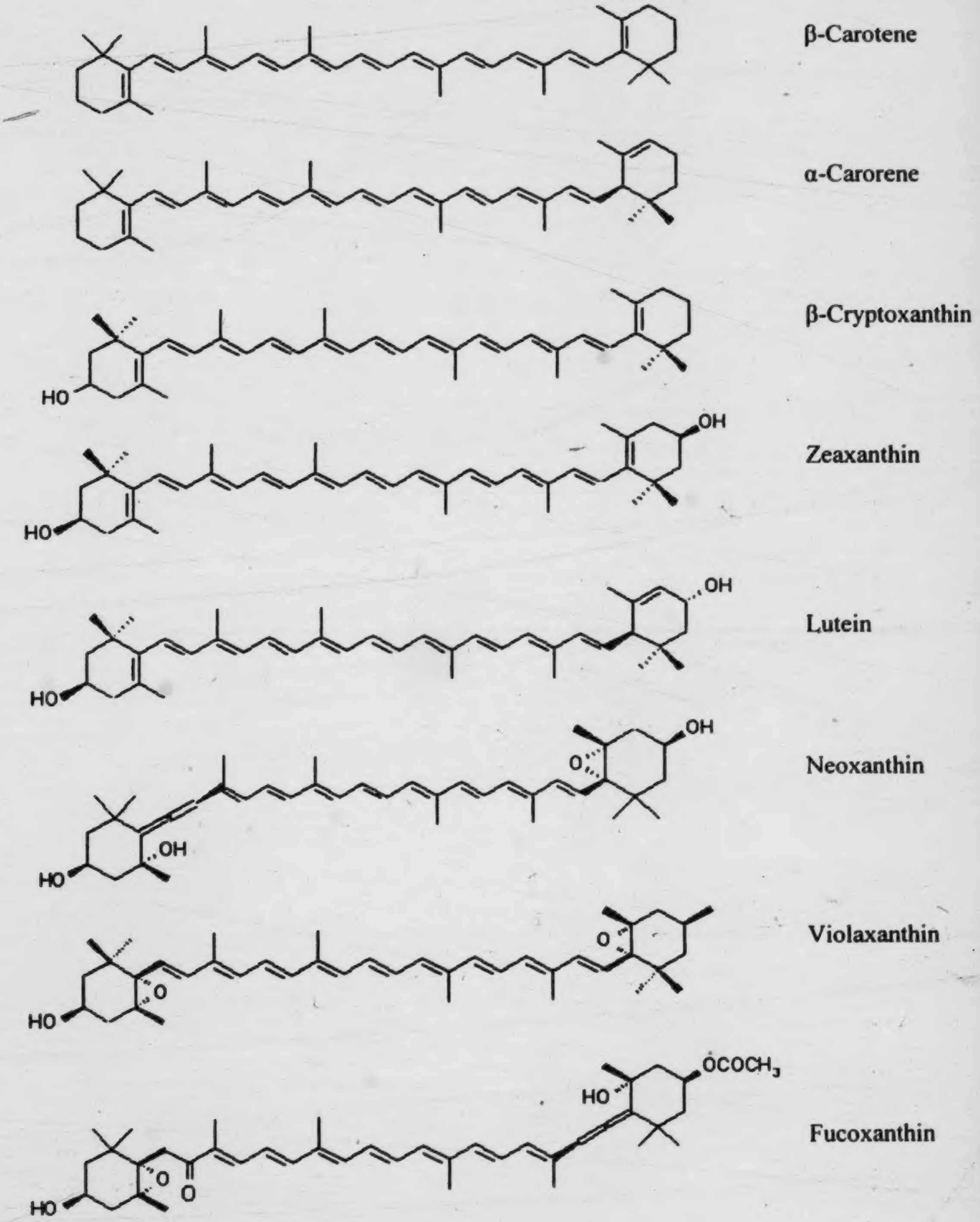

β-Carotene
α-Carorene
β-Cryptoxanthin
HO
Zeaxanthin
OH
HO
Lutein
OH
HO
Neoxanthin
OH
O
OH
HO
Violaxanthin
O
O
HO
Fucoxanthin
OCOCH₃
HO
O
O
HO

Figure 1.3. Structures of important carotenoids

The melting points of carotenoids are usually fairly high and tend to increase with increasing molecular weight and functional groups. For example, β-apo-8'-carotenal, β-carotene, and canthaxanthin have melting points of 136-140 °C, 170-182 °C, and 208-210 °C, respectively (Tee, 1992).

Carotenoids can crystallize in a variety of forms and the color of the crystals vary from deep red through violet to almost black. The melting points of carotenoids are usually fairly high and tend to increase with increasing molecular weight and functional groups. The light absorption of carotenoids is in the wavelength range of 400-500 nm.

1.2.3.2. Chemical characteristics

Due to the presence of a highly reactive, electron-rich long system, carotenoids are susceptible to light, oxygen and heat, which are responsible for the instability of carotenoids.

1.2.3.2.1. Light exposure

A group of competing reactions takes place when carotenoids are exposed to light. Photoisomerization reactions compete with photodegradation reactions. The predominant reaction will depend on the temperature, light intensity and the presence of additional catalysts. Pesek and Warthesen (1990) studied the photoisomerization and photodegradation of *all-trans*-β-carotene samples, which were stored at 28 °C under 250 ft-c (footcandle) of light for various periods of time. They found that the photodegradation reactions were predominant.

1.2.3.2x2. Oxidation

Carotenoids, even in the crystalline state, tend to auto-oxidize and may break down rapidly if samples are stored in the presence of even trace amounts of oxygen. The usual indication of carotenoid break down is bleaching, i.e. loss of color due to the break down of the chromophore. In vivo, the carotenoids are usually stabilized to a considerable degree by proteins and other molecules in their immediate vicinity. However, even in vivo, the carotenoids are still susceptible to oxidative damage if they become exposed to oxidizing species or free radicals. Easily being oxidized, on the contrary, is the important feature of carotenoids as antioxidants. Since the electron density is higher at the terminal double bonds, the initial oxygen attacking site on the carotenoids occurs at the terminal (EL-Tinay and Chichester, 1970).

1.2.3.2.3. Thermal degradation

At room temperature or at temperatures even up to about 100 °C, it is generally accepted that β-carotene can degrade by oxidation. At higher temperatures, however, oxygen concentration becomes less important than the effect of heat. β-Carotene is fairly unstable at elevated temperatures. Jideani (1992) studied the progressive loss of β-carotene in palm oil when heated to 160, 180, and 200 °C and held at these temperatures for 20, 40, and 60 min. It was found that the destruction was higher at 200 °C, where the β-carotene content was reduced by more than 85% in the first 20 min followed by a more gradual reduction. At the lower temperature of 160 °C for 20 min, the β-carotene content was reduced by 35%. The destruction rate of β-carotene by heat doubled for every 20 °C increase in temperature (Jideani, 1992).

1.2.4. Functions of carotenoids

1.2.4.1. Function as a colorant

Among nature's pigments, the carotenoids are the most widespread and probably have the most varied functions; of which one of the most obvious is serving as a class of food colorant ranging from light yellow to dark red. The color property of carotenoids is determined by its long system of highly delocalized π-electrons. The energy needed to excite an electron in the system corresponds to the light in the visible region in the wavelength range of 400-500 nm, which imparts carotenoids yellow, orange and red color. The color differences of carotenoids arise from the differences in the number of conjugated bonds. The yellow carotenoids absorb near the blue end of the spectrum, and as the number of double bonds, or functioning groups containing double bonds, increases, the absorption maxima shift towards the red end of the spectrum.

Pigments used as food additives can be classified according to their origin as natural or synthetic. According to the regulations set by the U.S. Food and Drug Administration, all the synthetic pigments are certifiable color additives and only seven certifiable colors are approved for use in food manufacturing; while pigments derived from natural sources such as annatto extract, dehydrated red beet, caramel, carrot oil, paprika oleoresin, saffron and oil of corn endosperm are exempt from certification for food use (Delgado-Vargas et al., 2000).

1.2.4.2. Function as pro-vitamin A

The importance of carotenoids in foods goes beyond their role as natural pigments. Various biological functions have been increasingly attributed to these compounds (Table 1.2). The best established function of carotenoids is their provitamin A activity, especially of β-carotene.

The conversion of β-carotene to retinol was documented through central and random cleavage (Nagao et al., 1996). In the central cleavage, oxygen reacts with the central two carbon atoms of β-carotene with the aid of β-carotene 15, 15'-dioxygenase,

Table 1.2. Some biological functions of carotenoids

Functions	Carotenoids	References
Provitamin A activity	β-Carotene α-Carotene β-Crypoxanthin	Van Vliet et al. (1996)
Antioxidant activity	All carotenoids	Palozza and Krinsky (1992)
Cell communication	β-Carotene Canthaxanthin Cryptoxanthin	Stahl and Siess (1998)
Immune function enhancers	β-Carotene	Lindley (1998)
UV skin protection	β-Carotene Lycopene	Solomons and Bulux (1997)
Macula protection	Lutein Zeaxanthin	Seddon et al. (1994)

followed by the cleavage of the central double bond of carotene to yield two molecules of *all-trans* retinal (Goodman et al., 1966; Goodman et al., 1967). In the random cleavage, one molecule of *all-trans* retinal was formed through a sequential set of β-apocarotenals, together with a variety of smaller fragments (Ganguly and Sastry, 1985).

On a world wide basis, about 60% of dietary vitamin A is estimated to come from provitamins (Simpson, 1983). Provitamin A has the advantage of being converted to vitamin A only when needed by the body; thus, avoiding potential toxicity from an overdose of vitamin A. For vitamin A activity in mammals, it is now known that a provitamin A carotenoid compound must have at least one unsubstituted β-ionone ring with an attached polyene side group. The other end of the molecule may vary in cyclic or acyclic structure and can be lengthened but not shortened to less than an 11 carbon polyene fragment.

Approximately 50 of the 600 carotenoids in nature have provitamin A activity (Olson and Krinsky, 1995). Among the various vitamin A precursors, *all-trans*-β-carotene has two β-ionone rings and therefore has the highest (theoretically 100%) vitamin A activity (6 μg β-carotene = 1 μg vitamin A). Some other carotenoids, containing only one β-ionone ring, such as *all-trans*-α- and γ-carotene, have only approximately half the biological value of *all-trans*-β-carotene. Phytofluence, ζ-carotene and lycopene, which are devoid of β-ionone ring, and lutein, zeaxanthin, violaxanthin and astaxanthin, in which both β-rings have hydroxyl, epoxy or keto substituents, do not have provitamin A activity.

1.2.4.3. Function as antioxidants

Antioxidant activity is another important biological function of carotenoids. Carotenoids have been shown to be effective antioxidants in vitro, but the situation in vivo is less clear. The antioxidant function of carotenoids is shown through the role they play in the free radical reactions and quenching singlet oxygen.

It has been proposed that free radicals can damage molecular lipids, proteins and DNA of the cells by chain reaction, therefore resulting in aging and death (Beckman and Ames, 1998). Depending on the tissue they attack, free radicals can cause different kinds of chronic diseases. If the attack is in the lung, they can cause lung cancer; in the blood and blood vessels, cardiovascular disease; in the eye, cataracts.

Being highly unsaturated, carotenoids can extract or donate electrons to or from suitable coreactants, leading to free-radical anions and cations, which in turn can react with oxygen and other molecules. Carotenoids therefore show both antioxidant and prooxidant activities under various conditions. Miller et al. (1996) evaluated carotenoid antioxidant activity against radicals and established the following order: lycopene > β-cryptoxanthin > lutein = zeaxanthin > α-carotene > echineone > canthaxanthin = astaxanthin. It was concluded that antioxidant activities are influenced by polarities that are increased with the presence of functional groups in the terminal rings.

As free radicals, some excited-state species such as singlet oxygen can also cause various diseases in the human body. Carotenoids can "quench" singlet oxygen by a physical mechanism, in which the excess energy of singlet oxygen is transferred to the carotenoid's electron-rich structure. The carotenoid is excited by this added energy into a "triplet" state (3Car*), and then relaxes into its ground state (1Car) by losing the extra energy as heat. Because this is a physical mechanism, the carotenoid structure is unchanged and the carotenoid remains to protect against further singlet oxygen. The carotenoids' efficacy as quenchers of excited species has been demonstrated in the treatment of human patients suffering from erythropoietic protoporthyria, a rare disorder in which singlet oxygen is produced via sensitization of free porphyrins accumulated in the skin (Mathews-Roth, 1981).

1.2.4.4. Special functions of xanthophylls in eyes

The xanthophylls, lutein and zeaxanthin, are the major carotenoids present in the macula region of the retina. Lutein and zeaxanthin represent approximately 36% and 18% of the total carotenoid content of retina, respectively (Landrum and Bone, 2001). They may play a major role in the prevention of age-related macular degeneration (AMD) and cataract.

Age-related macular degeneration is the leading cause of irreversible blindness in adults and is the result of degenerative changes that occur in the central region of the retina and the macula. These changes eventually lead to the loss of vision. Seddon et al. (1994) concluded from their eye disease case-control study that a high dietary intake of carotenoids was associated with a lower risk of AMD and two specific carotenoids, lutein and zeaxanthin, were more strongly associated with a reduced risk for AMD. Lutein and zeaxanthin are thought to function as a blue light filter and reduce the level of high-energy blue light reaching the most delicate retinal structures, which is thought to be capable of inducing retinal damage (Snodderly, 1995).

Cataract is clouding of the lens of the eye and/or of its surrounding transparent membrane, which obstructs the passage of light. This can also lead to blindness. Cataracts become more common with increasing age. It has been suggested that dietary antioxidants play a crucial role in the prevention of the oxidation of lens proteins and thus the formation of age-related cataracts (Jaques and Chylack, 1991). In a study involving 23 women with 55-78 years of age, Hammond et al. (1997) observed a significant inverse relation between macular pigment density and lens density, suggesting that lutein and zeaxanthin concentrations may be a suitable marker for lutein and zeaxanthin in the lens, which in turn may retard age-related increases in lens density. It has been hypothesized (Hammond et al., 1997) that oxidative damage to lens proteins and lipids present in the lens epithelium may be a contributing factor for the development of cataractogenises. The intake of carotenoid antioxidants may prevent its development by blocking oxidative changes to the lens proteins or by preventing lipid peroxidation within the epithelium of the lens (Jacques and Taylor, 1991).

1.3. DIETARY FIBRE

Dietary fibre (DF) is mainly the remnants of plant components resistant to hydrolysis by human alimentary enzymes. They are only naturally present in foods derived from plant sources; fruits, vegetables and grains. Foods of animal origin contain no fibre, even though the muscle meats are fibrous in the usual physical sense.

1.3.1. Historical perspective and definition of dietary fibre

Thought to be protective against toxemia during pregnancy, "*dietary fibre*" was first coined by Hipsley in 1953 to describe the unavailable carbohydrate content of plant foods (Asp, 1995). During 1950-1970s', the contrasts in the consumption patterns of DF between people in the developed and underdeveloped countries and the apparent relationship of certain diseases to fibre deficiency in the developed world was first documented by Cleave, the "father of the dietary fibre hypothesis" (Prosky, 2000). Following Cleaves's hypothesis, Trowell, Burkitt, Walker, and Painter adopted the term "dietary fibre" in conjunction with a number of health related hypotheses, which are referred to as "dietary fibre hypotheses' (The Dietary Fibre Definition Committee, 2001).

Since its first use, the terminology and definition of DF have continuously evolved and currently, as defined by The Dietary Fibre Definition Committee (2001), "dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibre promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation."

1.3.2. Chemistry of dietary fibre

Dietary fibre is composed of a number of complex and highly variable chemical compounds. Each of these compounds has a unique chemical and structural arrangement, which imparts its specific physiochemical, functional, and nutritional properties. Dietary fibre components are found in or are associated with the cell walls of plants. Some of these components are part of the intracellular cement. Others are produced by the plant in response to injury or to prevent seeds from desiccating. The composition and distribution of dietary fibre varies with plant source (Table 1.3). The knowledge of the fibre content, composition, and especially the monosaccharide composition and structural features is a key factor to understand the various physiochemical properties of these fibre compounds,

Table 1.3. Composition of dietary fiber in plant foods[1]

Fiber source	Noncellulose polysaccharides	Cellulose	Lignin
Cereals, 8 samples			
Average	75.7	17.4	6.7
Range	71-82	12-22	Tr-15
Raw vegetables, 11 samples			
Average	65.6	31.5	2.98
Range	52-76	23-42	Tr-13
Fruits, 10 samples			
Average	62.9	19.7	17.4
Range	46-78	9-33	1-38

[1] Adopted from Schneeman (1986)

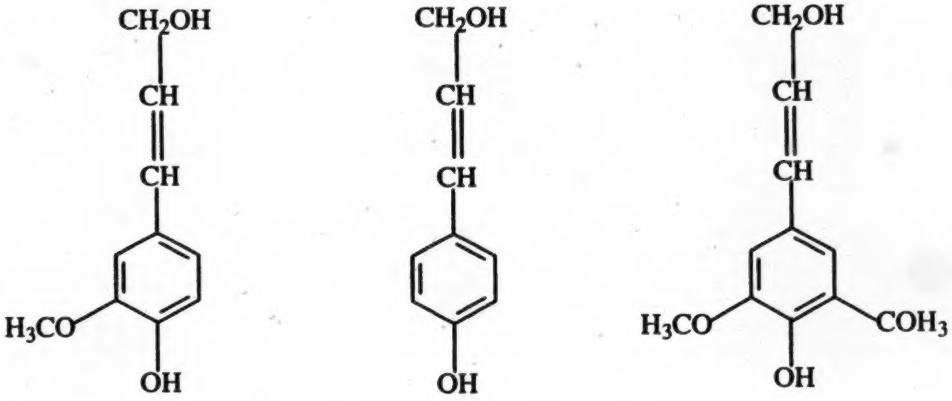
and therefore their impact on physiology and health. More detailed discussion of the individual dietary fibre components is given below.

1.3.2.1. Nonpolysaccharide dietary fibre

Lignin stands alone as a major fibre constituent since it is not a carbohydrate. Lignin is made up of aromatic polymers in plant cell walls and provides support to the plant structure by its "woody" characteristics. Lignin molecules are highly complex and variable polymers with a molecular weight usually between 1000 and 4000 Daltons (Cummings, 1976). Lignin is composed of three major aromatic alcohols: coumaryl, coniferyl, and sinapyl (Fig. 1.4). Other than providing mechanical support and thus allowing the plant to maintain its form, lignin can also conduct solutes and resist microbial degradation in plant cells. In animal alimentary tracts, lignin acts like a weak cation-exchange resin, for example, it is capable of combining with bile acids to form insoluble complexes, which are not absorbed.

1.3.2.2. Polysaccharide dietary fibre

Polysaccharides with 1-4 and 1-6 α-glucosidic bonding, or starches, are generally hydrolyzed by the amylase enzyme in the human digestive system. There are no enzymes in the mammalian small intestine, which are capable of hydrolyzing the β-1-4 linkage and thus this group of polysaccharides and some other nondigestible polysaccharides form the unavailable polysaccharides, namely, the non-starch polysaccharides (NSP). However, a portion of the starch can occur in a physical or chemical form that cannot be hydrolyzed by amylases either and is therefore not absorbed in the small intestine. This starch component has been referred to as resistant starch (RS), which physiologically has an action similar to DF. Non-starch polysaccharides are major components of DF, which



Trans-coniferyl *Trans*-p-coumaryl *Trans*-sinapyl

Figure 1.4. Structure of lignin aromatic alcohols

include the structural compounds such as cellulose, hemicellulose, pectin and β-glucan and other nonstructural compounds like gums and mucilages. Although it cannot be digested, some of the NSP may be fermented by bacteria in the colon and produce short-chain fatty acids and some gases like hydrogen, carbon dioxide, and methane.

1.3.2.2.1. Cellulose

Cellulose is probably the most abundant carbohydrate in nature and it has been defined as the skeletal "bones" in the plant kingdom (McCormick, 1985). It comprises 20 to 50% of the dry matter of many fibrous foods like vegetables and cereals (Spiller and Amen, 1975). It usually forms a complex matrix in plant cells by combining with hemicellulose, lignin and pectin. Cellulose has the simplest structure among all dietary fibre components. Cellulose is a linear polymer of glucose with β-1, 4 linkages (Fig. 1.5). It usually has a degree of polymerization (DP) ranging from 300 to 15,000 (Thernder, 1977; Dreher, 1987). The DP of cellulose molecule varies depending on the source of the cellulose and method of isolation. In the cell wall, consisting of several molecular chains, cellulose is present in the form of microfibrils, which make up macrofibrils. Like lignin, these fibrils have unusual mechanical strength and resistance to most chemicals and microorganisms (Theander, 1977). Cellulose is generally insoluble in water. However, the three dimensional arrangement of cellulose favors the formation of strong intermolecular and intramolecular hydrogen bonding. Therefore, cellulose can bind some water and hold the water throughout the alimentary tract.

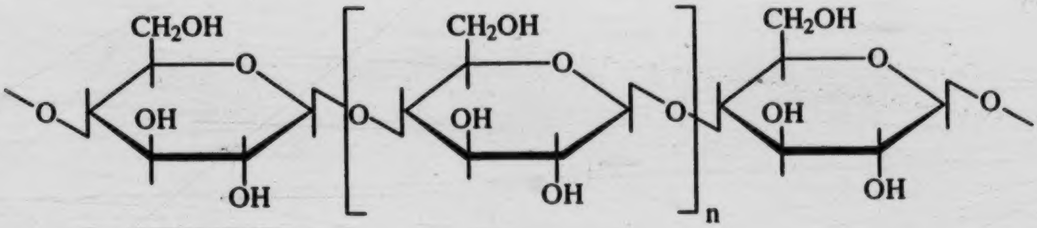

CH2OH
O
OH
OH
CH2OH
O
OH
OH
n
CH2OH
O
OH
OH
O
O
O
O

Figure 1.5. Structure of cellulose

1.3.2.2.2. Noncellulosic polysaccharides (NCP)

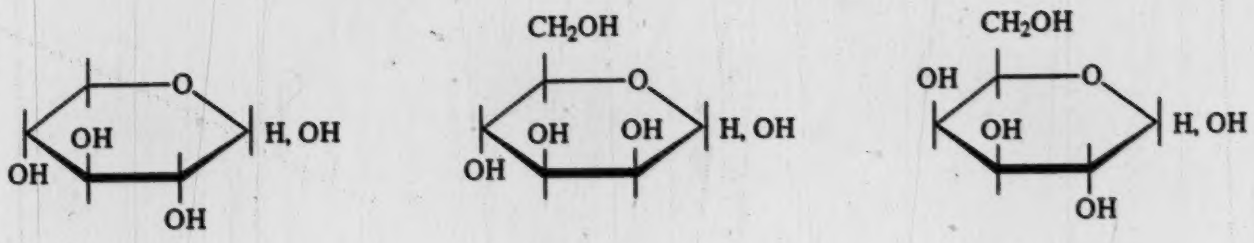
1.3.2.2.2.1. Hemicellulose

Hemicelluloses are a heterogeneous group of polysaccharides, which contain a variety of sugars in the polymeric backbone and side chains (Fig. 1.6). The DP of hemicellulose is much lower (50-200) than cellulose. They can be classified by the monosaccharide composition of the backbone, which includes xylans, glucomannans, and galactans. Soluble in alkali, hemicelluloses exhibit a wide range of solubilities, with a greater solubility being associated with a high degree of branching. The hemicelluloses are not hydrolyzed by intestinal enzymes, but are more or less readily broken down by bacteria to form compounds that may be absorbed and yield energy to the body. Bacterial action on hemicelluloses can produce appreciable quantities of short chain fatty acids, chiefly acetic, propionic and butyric acids.

1.3.2.2.2.2. Pectic substances

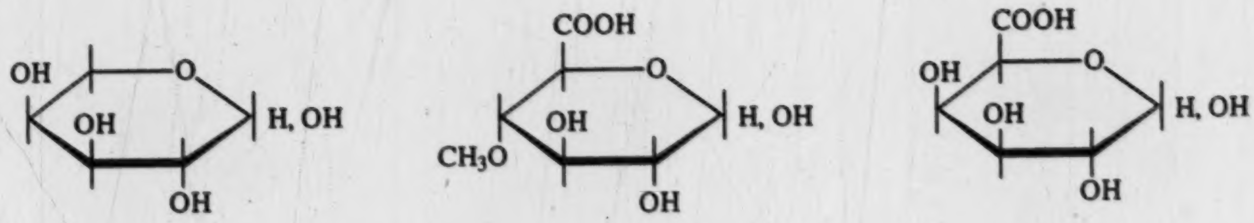
Pectic substances are a group of polysaccharides consisting mainly of α-1, 4 glycosidic linked D-galacturonic acids (Fig. 1.7) with rhamnose insertions at intervals as backbone. Radiating from the main chain are side chains consisting of certain sugars such as xylose, arabinose and galactose. The carboxyl groups on the galacturonic acids are often esterified by methyl or acetyl groups. The molecular weights of pectic substances are usually large, ranging from 50,000 to 150,000 Daltons, corresponding to from several hundred to about 1000 constituent units (Anonymous, 1984). Pectic substances are soluble in water and have gel forming properties.

Backbone chains



D-Xylose D-Mannose D-Galactose

Side chains



L-Arabinose 4-O-Methyl-D-glucuronic acid D-Glucuronic acid

Figure 1.6. Structure of sugars that make up hemicellulose

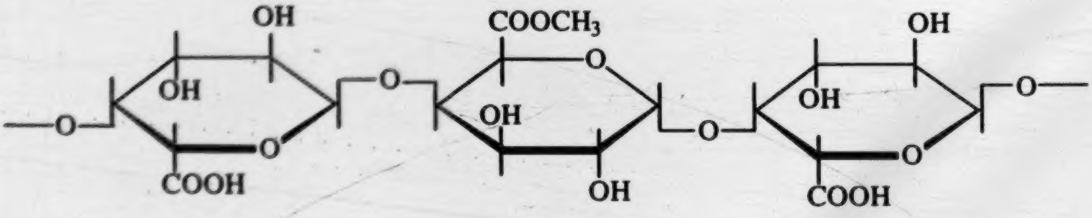

OH
OH
COOH
COOCH3
O
OH
OH
OH
OH
COOH
O

Figure 1.7. Structure of pectin

1.3.2.2.2.3. β-Glucan

β-Glucan is unbranched glucose polymer containing both β-1-3 and β-1-4 links in various proportions. The presence of β-1-3 linkage creates a kink in the molecular structure and therefore it is more soluble in water compared to cellulose. The typical ratio between (1, 4) and (1, 3) linkages of β-glucan is 2 and 3 to 1. The molecular weight of β-glucan ranges from 20,000 to 5,000,000 Daltons (Dreher, 1987).

1.3.2.2.2.4. Gums

Gums are obtained from seaweed, tree exudates, seeds, microbial fermentation, and modified celluloses. The plant exudates are produced by plants to seal off a damaged section and prevent invasion by microorganisms. To allow for large-scale or commercial production of gums, plants are usually intentionally injured. The monomeric units found in gums include the neutral sugars, uronic acids and other acid groups. In the food industry, gums are considered to be hydrophilic polymeric material that can be dissolved or dispersed in water to provide thickening, gelling, suspending, emulsifying, and film-forming properties to food.

1.3.2.2.2.5. Mucilages

Mucilages are the products of normal plant metabolism and are derived from barks, roots, leaves, seeds and even flowers. They are a mixed group of complex polysaccharides, which are not generally part of the cell wall. Mucilages are usually neutral polysaccharides.

1.3.3. Physical and physiological properties

A diet high in dietary fibre, especially high in fruits and vegetables, has some beneficial effects on human health, although it is not clear yet whether the benefits lie in the fibre itself or the high vitamins, minerals and phytochemicals present in fruits and vegetables and/or the low fat and calories. The physiological effects of dietary fibre on health cannot be separated from its physical properties, which are dictated by its chemical properties. Therefore, the possible mechanisms of dietary fibre on some specific health problems will be discussed along with its physical properties.

1.3.3.1. Solubility and effects on constipation and diverticulosis

The dietary fibre components are typically classified into soluble and insoluble fibres based on their water solubility. The fact that there are both soluble and insoluble fibres has tremendous impact on its physiological functions. Some important physiological effects are contributed by the fact that some fibre components are not dissolved in water.

Being not soluble in water but absorbing water, cellulose, hemicellulose and lignin are effective bulking agents. They contribute to the fecal bulk both by their presence as undegraded fibre residue and by increasing the water content of the fecal mass. On the contrary, the soluble fibres such as gums, mucilages and pectins are ineffective bulking agents largely due to the almost complete degradation via bacterial fermentation (Jalili et al., 2000). However, their fermentation contributes to the significant increase in bacterial cell mass.

Constipation is not a disease. It is a symptom of infrequent and/or difficult bowel movements. The large amount of soft stool produced from high dietary fibre intake makes the transit time short and therefore is effective for the prevention and alleviation of constipation. The relief of constipation on the other hand benefits the human body with less exposure of the intestinal wall to the toxic substances (Gordon, 1989).

The condition of diverticulosis is characterized by small out-pouches in the colonic wall. Normally, smooth muscle contraction is used to eliminate feces from the colon. During constipation conditions, muscular contraction results in greater pressure. Diverticulosis is thought to be a result of excessive pressure generated by the contractions of smooth muscle surrounding the colon. An out-pouching of colon wall, named diverticula, may develop under such pressure. An individual diagnosed with diverticulosis presents multiple diverticula. Diverticulosis normally does not lead to serious problems; however, when the diverticula become inflamed, it produces a painful and serious acute condition called diverticulitis. An increase in dietary fibre intake can relieve the problem by increasing the fecal bulk, and thus can reduce the pressure generated by muscular contraction of the colon, hence reduce the incidence of diverticulosis (Meister, 1996).

1.3.3.2. Fermentability and effect on colon cancer

Dietary fibre cannot be digested by the non-microbial enzymes in the mammalian small intestine. It is however fermentable to varying degrees of degradation within the large bowel. The degree of degradation varies considerably among the polysaccharides. Pectins, mucilages and gums appear to be completely degraded, whereas cellulose and hemicellulose are only partially broken down (Wildman and Medeiros, 2000). Furthermore, the extent of breakdown may be related to the physical structure of the plant. Fibre from fruits and vegetables appear to be more fermentable than that from cereals and grains (Jalili et al., 2000).

Other than accounting for a significant portion of the fecal mass and thus contributing to fecal bulk, the bacterial degradation of dietary fibre may also protect against colon cancer. The short chain fatty acid by-products of bacterial fermentation may influence the physiological response to fibre (Pomeranz, 1985). Butyrate has been found to induce differentiation and apoptosis (programmed cell death) in cancerous colon cells (Heerdt et al., 1994), therefore providing a potential mechanism for inhibiting multiplication of carcinogenic cells. Hague et al. (1995) found that, besides butyrate, acetate and propionate can also stimulate the apoptosis of carcinogenic cells, but among these three, butyrate was the most effective one. However, it seems confusing that butyric acid also appears to stimulate the growth and proliferation of normal colon cells (Ichikawa and Sakaka, 1998). The possible explanation for this apparent paradox between normal and cancerous cells is the fact that a cancerous cell may have a different response to butyric acid with its phenotype being altered. At the same time, the fermentation process may affect the colon cells by lowering the pH of the large bowel or by modifying the metabolic by-products of intestinal flora. However, as many studies examining fibre intake and colon cancer risk in the past few years have failed to find a protective effect (McKeown-Eyssen et al., 1994; MacLennan et al., 1995; Fuchs et al., 1999), the relationship between colon cancer and dietary fibre intake is not as clear as it was once thought and the role of dietary fibre in colon cancer prevention has become more difficult to assess.

1.3.3.3. Hydration capacity and effects on diabetes

Hydration properties of DF are of great importance due to its nutritional implications. The water-hydration property is strongly determined by the physicochemical properties and composition. Dietary fibres have a limited capacity to bind water, which is termed the saturation point or water binding capacity (WBC). The ability of the different fibres to bind water is largely attributed to the presence of sugar residues with free polar groups such as OH, COOH, and C=O. These polar groups allow the formation of hydrogen bonds with adjacent water molecules. Components such as pectic substances, mucilages, and to some extent hemicellulose tend to have high WBC, whereas cellulose and lignin tend to have low WBC.

Dietary fibre has been shown to modify the glycemic response of a food and therefore has been suggested to be useful in the diabetic diet (Wildman and Medeiros, 2000). The glycemic response is a measure of the ability of a food to elevate blood glucose level. Glycemic response can be estimated by glycemic index, which provides a measure of the rise in blood glucose levels following consumption of food products containing carbohydrates. Glycemic index is represented by a percentage of the value of the tested food to that of a reference food such as glucose, which has the highest value 100 (Table 1.4).

Foods with a high glycemic index are rapidly absorbed and cause a sharp increase in blood glucose whereas foods with a low glycemic index lead to a moderate increase in blood glucose. Many factors, which affect gastric emptying and transit time, can modify glycemic index of a particular food or a meal. The mechanism that fibre modifies the glycemic response has several modes of action. First, in the stomach, it can slow down the gastric emptying and thus reduce hunger and dietary intake. Second, both in the stomach and in the small intestine, it impairs and retards the contact of food with digestive enzymes. Third, the transport of released glucose to the absorption surface is slowed down (Edwards et al., 1988). Fourth, an unstirred layer is formed at the absorptive surfaces; therefore, glucose, as well as cholesterol, can pass only by a slow diffusion

Table 1.4. Glycemic index of some foods[1]

Highly glycemic foods		Moderately glycemic foods		Low glycemic foods	
Glucose	100	Orange Juice	57	Apple	36
Baked Potato	85	White Rice	56	Pear	36
Corn Flakes	84	Popcorn	55	Skim Milk	32
Cheerios	74	Corn	55	Green Beans	30
Graham Crackers	74	Brown Rice	55	Lentils	29
Honey	73	Sweet Potato	54	Kidney Beans	27
Watermelon	72	(Ripe) Banana	50	Grapefruit	25
White Bread	70-72	Orange	43	Barley	25

[1] Adopted from Anonymous (2003g)

process (Flourie et al., 1984; Lund et al., 1989). In humans, the glucose absorption has been shown to be reduced by 10% when intestinal viscosity was increased to 50 mPa-s by perfusing a pectin solution and was reduced by 23% when viscosity was 360 mPa-s (Flourie et al., 1984). However, it has become clear that only a large amount of very viscous soluble fibre can cause a substantial improvement in blood glucose control. Besides, the fibres must be consumed witl [illegible] naybe even physically mixed with food- in order to exhibit their effect (Nutta [illegible] Therefore, to achieve a meaningful improvement in blood glucose control, the types of fibre must be specified, the doses of consumption must be large, and the length of consumption period must be long enough. (Meister, 1996).

1.3.3.4. Oil binding capacity and effect on lipid absorption

The capacity of total dietary fibre to bind oils has not been extensively studied. However, there are some commercially available dietary fibre supplements such as chitosan (Anonymous, 2003c), which is claimed to bind lipid and control body weight. Chitosan is produced by hydrolyzing chitin, a polysaccharide naturally present in the shells of shellfish. Chitosan's lipid binding capacity is largely contributed by its specific molecular structure, with NH_2 in the sugar residue. Chitosan is soluble in the stomach acid and is positively charged once it is solubilized. The positively charged chitosan is a powerful emulsifier of lipids in the stomach. Other naturally occurring dietary fibres, which are either negatively charged or neutral, do not have lipid emulsification capability nor do they prevent the digestion and absorption of lipids. As food mass moves into the intestine, it forms a gel and the chitosan in it becomes insoluble as the pH increases. The gelation and subsequent insolubility of the chitosan complex encapsulate significant amounts of dietary lipids. This insoluble gel with its entrapped lipids is excreted as waste and therefore, prevents lipid absorption into the blood.

1.3.3.5. Organic molecule adsorption and effect on coronary heart disease

The fact that fruits, vegetables and grain products containing dietary fibre, particularly soluble fibre, may reduce the risk of Coronary Heart Disease (CHD) is one of the 10 health claims approved by the U.S. Food and Drug Administration (Heller et al., 1999). The function that dietary fibre reduces CHD is probably related to its ability of adsorbing organic molecules, for example, bile acids. Bile acid adsorption of fibre is measured in vivo as the ability to increase fecal bile acid and steroid excretion. The ability of certain fibres to increase fecal bile acid excretion has been related to plasma cholesterol-lowing effect (Kay and Truswell, 1977). Many large epidemiological studies such as the Nurse's Health study and the Scottish Heart Health study have shown a reduced risk for CHD in both men and women (Anderson and Hanna, 1999; Wolk et al., 1999; Todd et al., 1999). The principle mechanism of soluble fibre's cholesterol reduction effect is the prevention of re-absorption of bile acids in the small intestine, which causes an increased breakdown of cholesterol for bile acid synthesis. Lignin seems to be very effective in binding bile acids, as well as pectins and other acidic polysaccharides. In contrast, cellulose has little effect in bile acid binding (Schneeman, 1986).

Fibre has also been implicated in reducing the risk of CHD through mechanisms other than plasma cholesterol modification. One such example is through modification of blood clotting ability (Jalili et al., 2000). Enhanced blood clotting increases the risk of developing plaques in the coronary arteries. A plaque is a combination of cholesterol, other fatty materials, calcium and blood components that stick to the artery wall lining. As plaque builds up in an artery, the artery gradually narrows and can become clogged. This is the main cause of cardiovascular diseases. The blood clotting ability is dependent on fibrinogen levels and the quality of the resulting fibrin network. Fibrinogen is a soluble plasma protein that can form, upon interaction with thrombin, an insoluble fibrin network, which is the major structural and supporting element of a blood clot. Pectin has been found to influence the concentration and quality of fibrin networks in the blood and reduce the strength of these networks, thus reduce the risk for CHD (Veldman et al., 1997).

1.3.3.6. Cation exchange capacity and concerns on mineral absorption

The ability of dietary fibre to bind minerals such calcium, zinc and iron has commonly been referred to as the cation-exchange capacity (CEC) of dietary fibre (Gordon, 1989). This property of dietary fibre is not well understood. However, it was noted that the cation bridge between a mineral and fibre can serve as a mechanism where the positively charged cations can chemically bond to the generally negatively charged surface of dietary fibre (Anonymous, 2003d). The number of free carboxyl groups and the uronic acid content of dietary fibre play an important role in the cation exchange properties of fibres (Schneeman, 1986). Pectins tend to have high CEC, especially with the high content of unesterified uronic acid groups. Phytic acid is another component that is associated with dietary fibre, which has also been suggested to have a mineral binding effect. Concentrated fibre supplements, unlike fibre-rich food products, do not contain minerals and might have a negative effect on mineral availability (Meister, 1996).

1.3.3.7. Other physical properties

1.3.3.7.1. Particle size, density and pH

Particle size, density and pH are factors which influence some other physical and physiological properties of fibre such as solubility and water/oil binding capacity. Particle size and distribution not only influence the solubility, viscosity and gel forming properties of some soluble fibre, but also the water binding capacity of both soluble and insoluble dietary fibre (Gordon, 1989). Commercial grade pure cellulose and wheat bran tend to bind less water as the particle size is decreased (Stephen and Cummings, 1979). Typically, a decrease in particle size of dietary fibre is associated with increased density and surface area. The water binding capacity of dietary fibre is pH dependent, especially for charged polysaccharides (Gordon, 1989).

1.3.3.7.2. Color

Along with size, shape, and texture, color is an important factor that contributes to the basic appearance attributes of food. Food products with proper color have a direct effect in provoking people's appetite. So does the color of fibres used as a food ingredient. The addition of fibre to a food product may impart undesirable color. Therefore, a light colored fibre ingredient is required to avoid any color modification of the final product.

1.3.3.7.3. Antioxidant activity

Antioxidant activity is a unique characteristic of fibre from fruit and vegetable sources. The bioactive compounds such as carotenoids, flavonoids, and polyphenols in fruit and vegetable impart the fibre some level of antioxidant activity, which may enforce the physiological effect of fibre in the body.

1.3.3.7.4. Energy

Compared with the available carbohydrates, dietary fibre is indigestible and contributes only a small amount of energy due to the fermentation in the colon. Low energy contribution is another important property that makes dietary fibre useful in weight control.

1.3.4. Some functional properties of dietary fibre in food applications

The functional properties of dietary fibre are almost exclusively contributed by the soluble fibre components, which perform as stabilizers in emulsion, suspension and foam systems. Upon interaction with water, polysaccharides exhibit two basic properties, namely, viscosity and gelling properties, from which and together with interaction with the other molecules such as proteins and lipids, other useful functions in food are derived.

1.3.4.1. Viscosity

Viscosity is the basic rheological property which characterizes the flow behavior of food systems. With the long chain polymers dissolving or dispersing in water, the water-soluble fibre gives the solution a thickening or viscosity-producing effect. This thickening property is common to all gums and the degree of thickening varies between gums. Most gums give high viscosity at low concentrations; only a few gums give low viscosity at fairly high concentrations.

1.3.4.2. Gel formation

In spite of the fact that all gums thicken and impart viscosity to aqueous solutions, only a few gums have the property of being able to form gels. The gelling action can be described as the association or cross-linking of polymer chains to form a three-dimensional continuous network, which traps and retains the liquid within it to form a firm, rigid structure that has the ability to resist flow. The cross-linking of polymer chains is mainly due to the interactions between chains through hydrogen bonds. Some other bonds like ion-bridging, hydrophobic and van der Waals' forces are also contributing factors in specific conditions. Two or more chains may be attracted by the cross-linking bonds. As viscosity is the measurement of the thickness in liquid systems, gel strength or rigidity is the major parameter in gel systems.

1.3.4.3. Stabilization effect in food systems

In oil/water emulsion and suspension systems, hydrocolloids function as a stabilizer primarily by thickening and increasing the viscosity of the aqueous phase, so that it inhibits or minimizes the tendency of the dispersed oil globules to migrate and coalesce. Some gums such as gum arabic can also stabilize emulsions by means of a protective colloid action. The gum is adsorbed at the interface to form a coated particle, which then repels other particles and maintains a stable dispersed state. Some hydrocolloids can also perform as surfactants and reduce surface tension thus allowing easier formation and maintenance of stable emulsions.

Hydrocolloids are also very effective functional agents in making stable foam products. They can act as whipping agents to permit the aeration and formation of foams. As well, they can also act to stabilize the interfacial film and thus prevent the leakage of air and collapse of the structure.

Besides, the thickening effect of polysaccharides is very useful in film formation, encapsulation, and crystallization inhibition in the food industry. Cellulose ethers are effective in preventing oil absorption by forming a lipophobic film in fried foods such as potato slices and doughnuts (Glicksman, 1982). Ice and sugar are the two most important crystalline materials in foods. Their crystallization in ice cream and bakery icings can be controlled by the addition of hydrocolloids.

1.4. SUPERCRITICAL FLUID EXTRACTION

Extraction is one of the unit operations in a separation process. The basis of a separation is the property difference between components, which includes density, volatility, solubility, reactivity and polarity. In the extraction process, the basis of separation is solubility. A constituent of a solid or of a liquid mixture, which is more soluble than others is transferred to another selective liquid. In a solid-liquid extraction, the driving force of mass transfer is the concentration difference between the solute-bearing inert matrix and the solvent. The commercial instant coffee production is a solid-liquid extraction. Other industrial extraction applications include the extraction of oils from oilseeds, the extraction of essential oil and pigments from paprika, and the extraction of sugar from sugar beets.

Carotenoids have been extracted from various sources with ethanol, isopropanol, acetone, butanol, and hexane (Table 1.5). However, the usage of these solvents in industrial scale has been restricted because of the strict government regulations and increasing consumer demand for "natural" products. On the contrary, there is one unique group of solvents, supercritical fluids, that is gaining increasing interest.

1.4.1. Supercritical fluids and their properties

A supercritical fluid (SCF) is one phase of matter in between a liquid and a gas. In a typical phase diagram (Fig. 1.8), along the boiling curve, with an increase in temperature and pressure, the coexisting liquid becomes less dense because of the

Table 1.5. Solvent extraction of carotenoids

Matrix	Carotenoids	Solvent	References
Alfafa	Overall	Acetone Isopropanol n-Butanol	O'Day and Rosenau (1982)
Crawfish waste	Astaxanthin	Soybean oil	Meyers and Chen (1985)
Green algae	Astaxanthin	Acetone	Kobayashi et al. (1997)
Chili Guajillo puya (*Capsicum annuum L.*)	Overall	Ethanol	Santamaria et al. (2000)
Marjoram (*Origanum majorana L.*)	β-Carotene Lutein	Hexane Ethanol	Vagi et al. (2002)

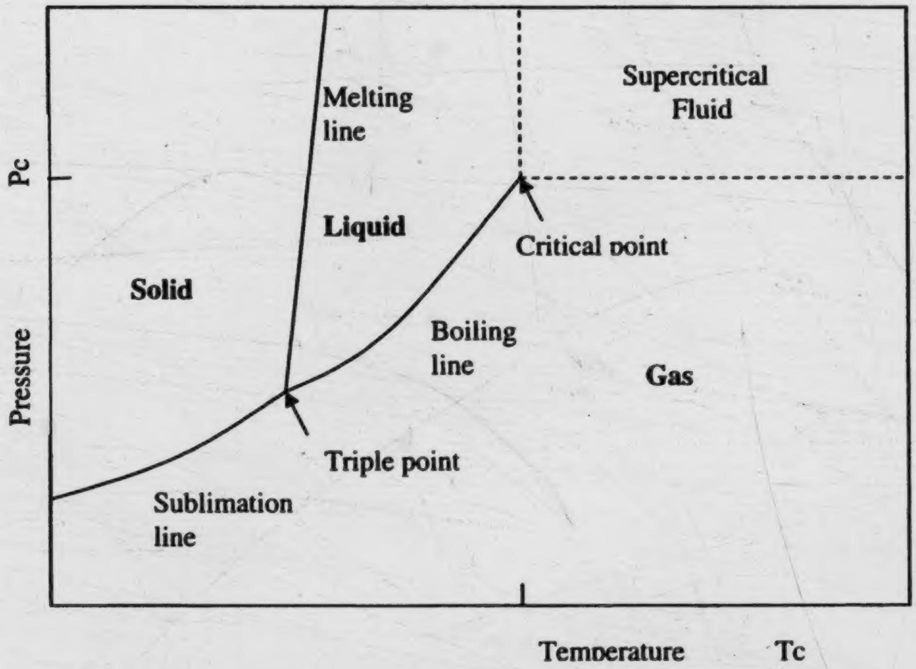

Pc
Pressure
Melting line
Supercritical Fluid
Liquid
Critical point
Solid
Boiling line
Gas
Triple point
Sublimation line
Temperature
Tc

Figure 1.8. Pressure-temperature phase diagram for a pure component

thermal expansion and the coexisting gas becomes denser because of the compression. Eventually, the densities of the two phases become identical and the distinction between gas and liquid disappears at the critical point. The substance in the region where the temperature and pressure are above the critical point is a supercritical fluid. The temperature at the critical point is critical temperature, which is the highest temperature a gas can be converted to a liquid by an increase in pressure. The pressure at the critical point is critical pressure, which is the highest pressure a liquid can be converted to a gas by an increase in temperature. Each substance has its own critical temperature and pressure. The critical properties of some substances are listed in Table 1.6. When liquids or gases are converted to supercritical fluids, there is no sudden physical property change. So, there is no phase transition line like the sublimation, boiling and melting lines. The dashed lines in Figure 1.8 indicate the critical temperature and pressure.

In the supercritical region, the molecules of the component cluster in local sites, which makes a supercritical fluid to have much higher local density than the bulk density (Bunker et al., 2002). This character that a supercritical fluid has different local and bulk densities imparts unique physicochemical properties, which are intermediate between those of liquids and gases. The clustering of the molecules gives supercritical fluids high density, which is similar to that of a liquid, whereas the distances between clusters give supercritical fluids low viscosity and high compressibility and diffusivity, which are like those of a gas (Table 1.7). Behaving like a gas, SCF can also expand and be mixed with other gases.

The combined liquid-like solvating characteristics and gas-like mass transfer properties make a supercritical fluid able to penetrate substances rapidly to dissolve the

Table 1.6. Critical properties of typical supercritical solvents[1]

Solvent	Critical properties		
	Temperature (°C)	Pressure (MPa)	Density (g/cm^3)
Carbon dioxide	31	7.37	0.47
Ethane	32	4.88	0.20
Nitrous oxide	36	7.25	0.45
Hexane	234	2.97	0.23
Isopropanol	235	4.76	0.27
Methanol[2]	240	7.90	0.27
Ethanol	243	6.38	0.28
Toluene	318	4.12	0.29
Water	374	22.06	0.32

[1] Adopted from Hoyer (1985)
[2] Adopted from Hawthorne (1990)

Table1.7. Properties of gas, liquid and supercritical fluid[1]

Properties	Phase		
	Gas	SCF	Liquid
Density (g/cm^3)	$(0.6\text{-}2.0)\times 10^{-3}$	0.2-0.9	0.6-1.6
Diffusivity (cm^2/sec)	0.1-0.4	$(0.2\text{-}0.7)\times 10^{-3}$	$(0.2\text{-}2.0)\times 10^{-5}$
Viscosity (g/cm sec)	$(1\text{-}3)\times 10^{-4}$	$(1\text{-}9)\times 10^{-4}$	$(0.2\text{-}3.0)\times 10^{-2}$

[1] Adopted from Hoyer (1985)

solute and transfer the solute to the bulk solvent at a high rate. However, unlike a liquid, which has constant solvent power, a supercritical fluid has adjustable solvent power or density, which is affected by temperature and pressure. Like density, the viscosity and diffusivity are also temperature and pressure dependent. The diffusivity of a supercritical fluid increases with temperature, whereas, the viscosity decreases (unlike a gas) with temperature. Especially, in the region near the critical point, relatively small changes in temperature or pressure will produce large changes in density, diffusivity and viscosity. The temperature and pressure effects on the density is clearly shown in Figure 1.9, reduced pressure ($P_r = P/P_c$) versus reduced density ($\rho_r = \rho/\rho_c$) at various isotherms (T_r is reduced temperature and $T_r = T/T_c$). In the region near the critical pressure of the solvent, as the temperature increases, the density of the solvent decreases rapidly. However, in the high pressure region, the density decrease with temperature is much smaller. The effect of pressure on density change is illustrated by the slope of the isotherms. The flatter the slope, the greater the density change with pressure.

Another important characteristic of a supercritical fluid is that it presents no surface tension or wetting problems. This allows a supercritical fluid to easily penetrate the matrices with pores and thus enhances the extraction efficiency.

1.4.2. Brief history of supercritical fluids and technology

The critical point of a substance was discovered by Baron Cagniard de la Tour in 1822 and for many years after that, the critical point was referred to as the Cagniard de la Tour point (McHugh and Krukonis, 1994). The supercritical behavior of carbon dioxide and its critical value was reported by Thomas Andrews in 1869 (McHugh and Krukonis, 1994). The power of a supercritical fluid to dissolve low-vapor-pressure solid materials

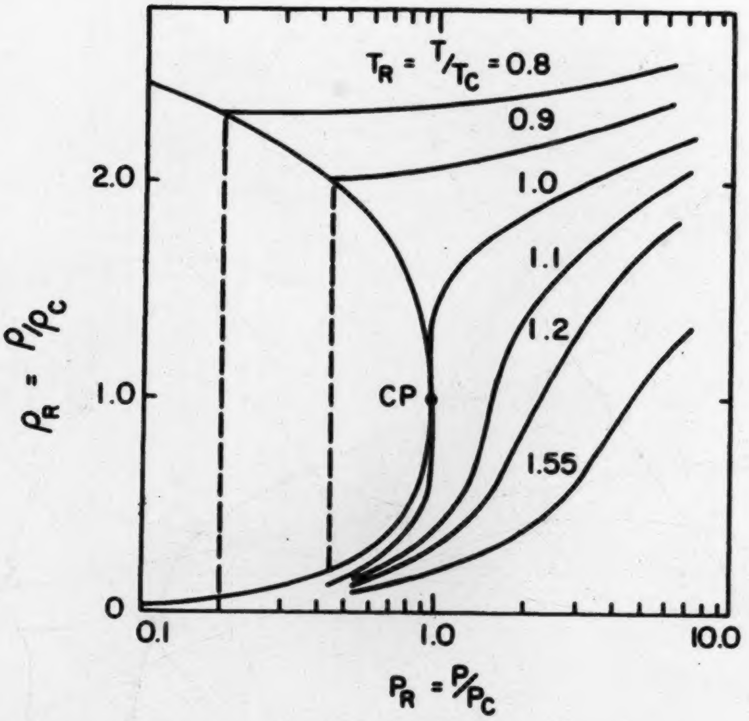


Figure 1.9. Reduced pressure against reduced density at different isotherms for carbon dioxide (McHugh and Krukonis, 1994)

was first published by Hannay and Hogarth (1879). In 1974, the application of supercritical carbon dioxide extraction to natural food products was first introduced by Kurt Zosel in coffee decaffeination (Anonymous, 2003e). Since then, a large number of commercial supercritical carbon dioxide extraction plants related to food processing have been built, starting with a coffee decaffeination plant built by HAG A. G. in West Germany in the early 1980's (currently owned by General Food Corp.) (Anonymous, 2002).

1.4.3. Supercritical fluid extraction

The areas of application for supercritical fluids are numerous and include extraction, chromatography, material synthesis, chemical reactions, polymerization, particle formation, textile dyeing and dry cleaning. Extraction was probably the earliest application of supercritical fluids. Supercritical fluid extraction has developed very rapidly in the last decade and probably is the greatest one among all the possible applications of supercritical fluids today.

The basic extraction process is shown in Figure 1.10. To perform the extraction, the most commonly used carbon dioxide is compressed to a selected pressure and its temperature is adjusted to the desired level to achieve a supercritical state. As the supercritical fluid passes through the extractor, which is loaded with the feed material, it dissolves and extracts the soluble component. The solute-laden supercritical solvent leaves the extractor and passes through the pressure reduction valve, where the pressure and dissolving power of the supercritical fluid is reduced. Thus, the extracted component is not dissolved in the solvent any more and precipitate in the separator. Reduction of pressure causes cooling of the fluid and so heating of the depressurization valve is

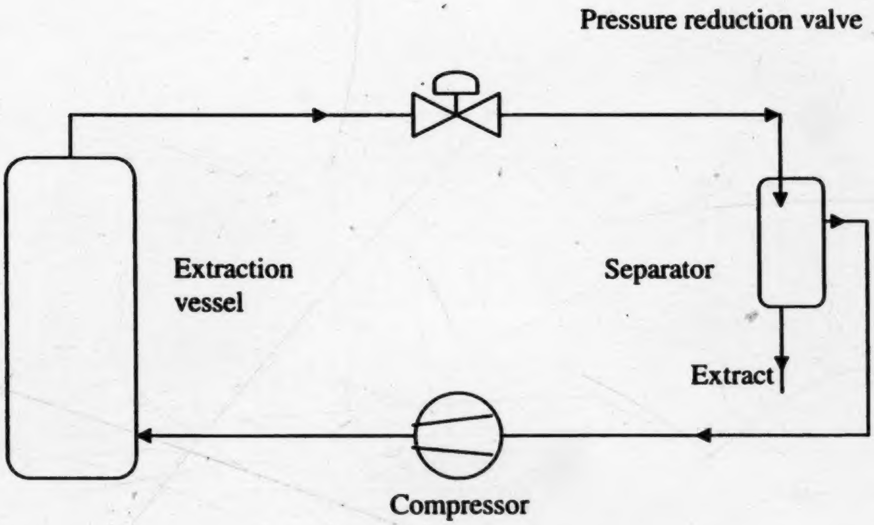

Pressure reduction valve
Extraction vessel
Separator
Extract
Compressor

Figure 1.10. Supercritical fluid extraction process

required to prevent freezing. The released carbon dioxide gas can be recycled for another extraction or vented. The separation of the extracted component from carbon dioxide can also be achieved by adjusting the temperature to minimize the solubility of the soluble component in the supercritical fluid.

It is postulated (Clifford, 1999b) that extracting a solute from a solid matrix with a supercritical fluid can be described as in Figure 1.11 by four steps: 1) rapid fluid entry into the matrix, 2) a reversible release process such as solute desorption from the matrix sites or penetration of a biological membrane, 3) solute diffusion to the external surface of the matrix particles, and 4) removal of solution by the bulk flow of the solvent. Therefore, the feasibility of an extraction is basically determined by three factors: the interaction of solute with the matrix, solubility of solute in solvent, and diffusivity of supercritical solution from interior to the surface of the matrix.

The extraction can be carried out in a static, dynamic or coupled static/dynamic mode. In a static extraction, a fixed amount of supercritical fluid interacts with the matrix and solute, as in the case of a cup of hot water and a tea bag. On the other hand, in a dynamic extraction, fresh supercritical fluid passes through the sample matrix continuously, as is hot water in a coffee maker. In the static and dynamic combined mode, a static extraction is performed for a certain period of time, followed by a dynamic one. This extraction mode is gaining popularity and is very useful for the situation where a solute must diffuse to the matrix surface to be extracted.

Dynamic extraction is the most easily modeled extraction mode. Figure 1.12 shows the theoretical extraction profile of a solute from a solid matrix in a dynamic extraction system. The initial extraction (region 1) occurs quickly and depends on the

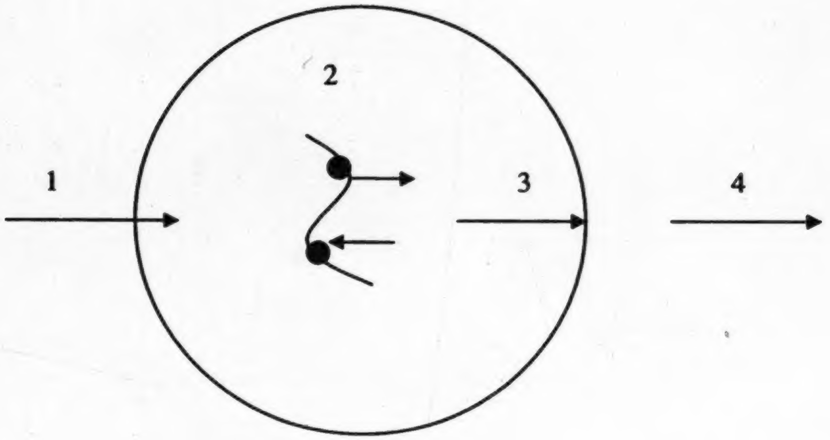


Figure 1.11. Steps in an extraction process

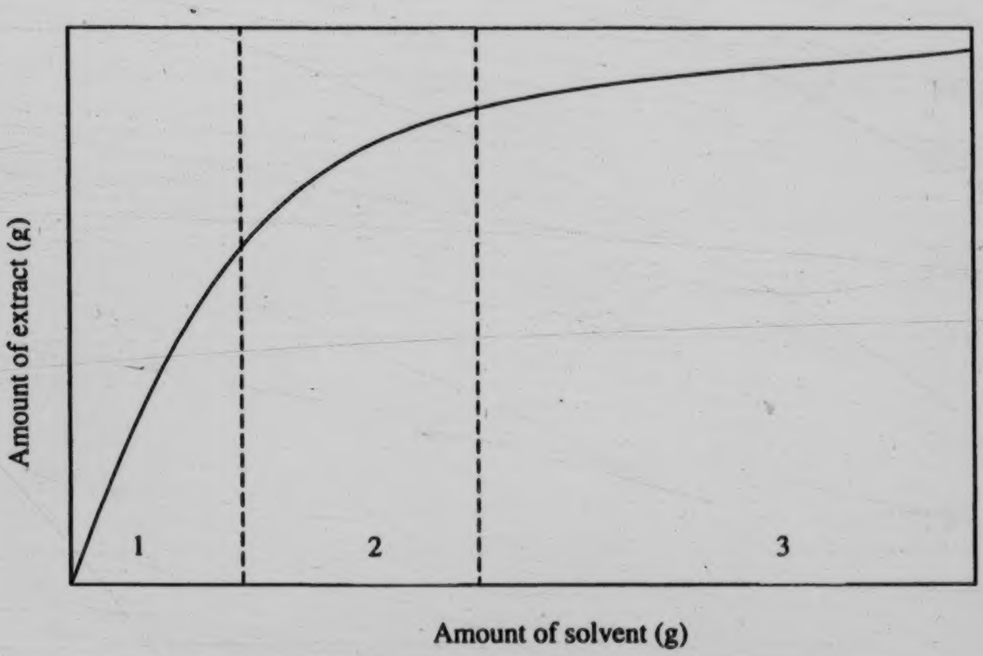


Figure 1.12. Theoretical dynamic extraction profile of a solute from a solid matrix

solubility of the bulk solute in the SCF and the movement of SCF throughout the system. During the initial period, the solute present on the external surfaces of the matrix is solubilized and purged out of the extraction vessel. This is followed by an enthalpically controlled extraction process (region 2), in which the solute-matrix interaction is disrupted. A transition to the diffusion-controlled process also occurs at this stage (region 2), during which the rate of extraction drops rapidly. The rapid decrease in the extraction rate may be caused by the depletion of the continuous layer of solute on the matrix surfaces thereby resulting in a reduced effective area available for solute transfer and hence a reduced overall mass transfer coefficient. When all the solute on the surface has been removed, the extraction rate depends on the diffusion of solute from the interior to the surface of the solids (region 3). This process is truly diffusion limited, which is controlled by the limited mobility of solute within the sample matrix. This rate is very low compared with the initial extraction rate.

1.4.4. Supercritical carbon dioxide extraction and factors affecting extraction

Although there is a wide range of supercritical fluids with different polarities, supercritical carbon dioxide (SC-CO_2) is the solvent of choice for most food applications. Supercritical ammonia is an attractive solvent for polar components; however, it is chemically reactive and tends to dissolve pump seals and stainless steel tubings. Supercritical methanol is also an excellent solvent, but because of its high critical temperature, it is a liquid at ambient conditions and therefore needs a complicated sample separation process after extraction. Nitrous oxide is an oxidizing agent and has the potential for explosions. Propane is flammable and requires additional safety precautions.

Carbon dioxide has a moderate critical pressure (7.3 MPa), which minimizes the compression costs and a low boiling point (-78.5 °C), which makes CO_2 easily removable after extraction. It is also an inert gas that is neither toxic nor flammable. It is available in abundance at high purity and low cost. With its low critical temperature (31 °C), carbon dioxide is an ideal solvent for extracting thermally labile compounds. Products extracted with SC-CO_2 have no chemical residue and are free of solvent.

1.4.4.1. Temperature and pressure

The solvating power of SC-CO_2 changes with density, which is modified by temperature and pressure. At low densities (0.2-0.45 mg/mL), SC-CO_2 behaves as hexane and at high densities (0.6 mg/mL) as methylene chloride (McNally, 1996).

At a constant temperature, as pressure increases, the density of CO_2 increases (Fig. 1.9), which means the intermolecular mean distance of CO_2 decreases and specific interactions between solvent and solute increase. Near the critical pressure of CO_2, 7.3 MPa, solubility increases dramatically because of a rapid increase in density with increasing pressure. A further increase in pressure at constant temperature results in a modest increase in solubility because CO_2 density is increased at a modest rate. If pressure is increased to an extremely high value, the solubility would reach a maximum.

The effect of temperature on solubility is somewhat more complex. An increase in extraction temperature results in both increased solute vapor pressure and decreased CO_2 density (Fig. 1.9), and therefore a reduced solvating power of SC-CO_2 at constant pressure. In the low-pressure region, the decrease in density with temperature predominates, which results in an overall solubility decrease with temperature. In the high-pressure region, the vapor pressure increase with temperature predominates, which leads to an overall solubility increase with temperature. Consequently, lower temperature favors extraction at low pressures, while higher temperature favors extraction at high pressures. A change of temperature may show no apparent effect at an intermediate pressure. As a consequence, the solubility isotherms of a typical compound in SC-CO_2 plotted as a function of pressure cross each other, giving rise to the so-called cross-over effect. The solubility increases with decreasing temperature at pressures below the cross-over pressure and increase with temperature at higher pressure.

1.4.4.2. Addition of a co-solvent

The modification of the solvating power of SC-CO_2 by changing temperature and pressure through density is the first choice of strategy to increase the solubility of a target solute. The addition of a small amount of co-solvent (also referred to as modifier or entrainer) into SC-CO_2 like hexane, methanol, ethanol and isopropyl alcohol is another option. The co-solvents added to the supercritical fluid may improve the solubility of compounds to be extracted by changing the polarity of the solvent mixture or the relative volatility of component (Peter and Brunner, 1980). Besides, co-solvents may also improve the diffusion of solute and the matrix factor by competing with the solute molecules for the sorptive sites, reducing solute-matrix interactions and swelling the matrix (Clifford, 1999a).

Most of the studies reported on the SC-CO_2 extraction of carotenoids utilized co-solvents (most frequently, ethanol), which increased the extraction yields significantly (Table 1.8). Vega et al. (1996) obtained an approximately 30-40% recovery of carotene extracts from carrot in SC-CO_2 extractions with no ethanol addition, while 5 and 10% ethanol addition resulted in 50-60% recoveries. Similarly, Baysal et al. (2000) achieved

Table 1.8. Summary of supercritical carbon dioxide extraction of cartenoids reported in literature

Matrix	Carotenoids	Temperature (K)	Pressure (MPa)	Co-solvent	References
Carrots	β-Carotene α-Carotene Lutein	313, 323, 333	7.8-29.4	Ethanol	Goto et al. (1987)
Leaf protein concentrates (alfalfa)	Carotene Lutein	313	10, 30, 50, 70	----	Favati (1988)
Fermentation broths	β-Carotene	343, 353	101.3	Ethanol	Cygnarowicz (1990a)
Broccoli florets Carrots Collard greens Corn Kale Mustard green Squash Turnip greens Zucchini	β-Carotene α-Carotene	313	33.8	Ethanol Hexane Methylene chloride Water	Marsili and Callahan (1993)
Sweet potatoes	β-Carotene	311, 321	13.8, 27.6, 41.4	----	Spanos et al. (1993)
Carrot (*Daucus carota L.*)	β-Carotene α-Carotene	313, 323, 333	30, 40, 50	Ethanol	Barth et al. (1995)
Micoalgae (*Chlorella vulgaris*)	Astaxanthin Canthaxanthin	313, 328	20, 35	----	Mendes et al. (1995)
Carrot pulp	β-Carotene α-Carotene	313, 328, 343	20.7, 27.6, 34.5	Ethanol	Vega et al. (1996)

Table 1.8 (continued)

Matrix	Carotenoids	Temperature (K)	Pressure (MPa)	Co-solvent	References
Paprika (*Capsicum annuum L.)*	Capsanthin Zeaxanthin β-Carotene	323	35	----	Vesper and Nitz (1997)
Carrots	β-Carotene	330	25	----	Subra et al. (1998)
Paprika	Capsanthin Capsorubin Zeaxanthin β-Carotene β-Cryptoxanthin	313	13.8-48.2	Acetone Ethanol	Jaren-Galan et al. (1999)
Paprika	β-Carotene	313	33.8	2, 2-Dimethoxypropane Diethoxypropane Trithylothofomate	Weathers (1999)
Tomato paste waste	β-Carotene Lycopene	318, 328, 338, 348	20, 25, 30	Ethanol	Baysal et al. (2000)
Ripe tomatoes	β-Carotene Lycopene	313, 323, 333, 343, 353	17.2-27.6	Chloroform Ethanol Ethyl ether Hexane	Cadoni et al. (2000)
Pressed palm oil (*Elaes guineensis*)	Carotenes	318	25, 30	----	Franca et al. (2000)

Table 1.8 (continued)

Matrix	Carotenoids	Temperature (K)	Pressure (MPa)	Cosolvent	References
Marigold (*Tagetes erecta*)	Lutein diestera	313-328	CO_2 density 0.7-0.9 g/mL	Acetone Acetonitril Ethanol Isopropanol Methanol	Naranjo-Modad et al. (2000)
Crawfish shell	Astaxanthin	323-333	13.8-31	Ethanol	Charest et al. (2001)
Crab (*Callinectes sapidus*) shell waste	Astaxanthin	322-334	29.5-33.5	Ethanol	Felix-Valenzuela et al. (2001)
Microalgae (*Spirulina maxima*)	Carotene	293-343	15-18	----	Canela (2002)
Marjoram (*Origanum majorana L.)*	β-Carotene Lutein	313-333	10-40	----	Vagi (2002)

an approximately 25% recovery for lycopene and a 45% recovery for β-carotene from tomato paste waste during SC-CO_2 extraction without ethanol addition, whereas extraction with 5% ethanol addition resulted in a 55% lycopene and a 50% β-carotene recovery. The enhancement effects of ethanol on carotenoids recovery in those two studies were both temperature dependent and the enhancements were pronounced with temperature. Barth et al. (1995) found that the co-solvent effect of ethanol was also interfered by pressure. At low pressure (30.3 MPa), high ethanol concentration (10%) gave the highest α- and β-carotene yield at all temperatures. At moderate pressure (40.5 MPa), more carotenes were extracted with high ethanol concentration (10%) at 30 °C and 50 °C, while more were extracted with low concentration of ethanol (5%) at 40 °C. At high pressure (50.6 MPa), less carotenes were obtained with ethanol addition at all temperatures. The co-solvent effect of ethanol was also feed material dependent. When pure CO_2 and CO_2 with 1, 3 and 5% ethanol were used to extract carotenoids from carrot (Goto, 1987), the extraction rate increased with co-solvent for raw carrot, while there was no effect for freeze-dried carrots. The co-solvent effect varies with the type of chemical solvent as well. When ethanol, ethyl ether, chloroform and hexane were used as co-solvent in SC-CO_2 extraction of lycopene and β-carotene from ripe tomatoes, only chloroform gave satisfactory results, and to a lesser extent, hexane (Cadoni et al., 2000).

A co-solvent introduced into supercritical fluids especially for food applications should not be toxic. In nature, health benign co-solvents are very limited. Water and ethanol are two co-solvents of choice for polar substances targeting food applications. For non-polar solutes, a non-polar co-solvent may be added to enhance solubility. Vegetable oil is a potential co-solvent in SC-CO_2 for fat-soluble solutes but such an approach has not been reported previously. Canola oil was chosen for this research, thus more detailed information about canola oil is given below.

Canola oil is one kind of vegetable oil, which has many advantages over other types. Canola, the largest oilseed crop in Canada, is the name used originally in Canada to identify rapeseed cultivars of *Brassica napus* and *Brassica campestris* (now called *Brassica rapa*) that have been selectively bred to have low levels of erucic acid (C22:1) in oil (<2%) and low levels of glucosinolates in meal (<30 micromoles). Canola oil, like other vegetable oils, is composed of 94.4-99.1% triglycerides (TG), esters of one molecule of glycerol and three molecules of fatty acids, covering a range of molecular weights, with a complex mixture of minor components. The most common fatty acids in canola oil are those containing 16 or 18 carbons, including palmitic, stearic, oleic, linoleic and linolenic acids, which account for about 95% of the total fatty acids in canola oil (Table 1.9). The combination of fatty acids on the glycerol moiety is complex, with 3n amount of potential molecular species where n is the number of different fatty acids present in the oil. The predominant triglycerides in canola oil are triolein (OOO, ~25%), linolein-diolein (LOO, ~25%), linolenin-diolein (LnOO, ~10%), olein-dilinolein (LLO, ~10%), linonenin-linolein-olein (LnLO, ~10%) (Przybylski, 2001).

Although there are no reports on the use of vegetable oil as a co-solvent, canola oil is expected to increase the solubility of carotenes in SC-CO_2 based on the findings of other studies and the fact that carotenoids are fat-soluble compounds. First, the relative volatilities of components can be altered by the addition of a co-solvent and the solubility of the nonvolatile components in a compressed gas increased with the concentration of co-solvent (Peter and Brunner, 1980). Second, it was observed that the solubility of a

Table 1.9. Fatty acid composition of canola oil[1]

Abbrevation	Common name	Systematic name	Melting point (°C)	Percentage in oil (w/w)
C14:0	Myristic	Tetradecanoic	54.4	0.1
C16:0	Palmitic	Hexadecanoic	62.9	3.5
C18:0	Stearic	Octadecanoic	69.6	1.5
C20:0	Arachidic	Eicosanoic	75.4	0.6
C22:0	Behenic	Docosanoic	80.0	0.3
Total saturated				6.0
C16:1	Palmitoleic	9-Hexadecenoic	----	0.2
C18:1	Oleic	9-Octadecanoic	16.3	60.1
C20:1	Gadoleic	9-Eicosanoic	----	1.4
C22:1	Erucic	13-Docosanoic	33.4	0.2
Total monounsaturated				61.9
C18:2	Linoleic	9, 12-Octadecanoic	-6.5	20.1
C18:3	Linolenic	9, 12, 15-Octadecanoic	-12.8	9.6
Total polyunsaturated				29.7

[1] Adopted from Przybylski (2001)

particular solid component in the ternary system of SC-CO_2, target solute, and a co-solvent or an impurity substance was considerably higher than that in the pure solid-SC-CO_2 system at the same temperature and pressure (Lee et al., 1988). Bamberger et al. (1988) measured the solubility of the quaternary system containing trilaurin (LLL), trimyristin (MMM), tripalmitin (PPP), and CO_2, and the ternary systems containing LLL-MMM, LLL-PPP, or MMM-PPP with CO_2, as well as the solubilities of the individual pure triglycerides at identical conditions. Their data suggested that the solubilities of the less volatile lipid components in these systems were significantly enhanced in the presence of the more volatile triglyceride species in the system and that the solubility enhancement was stronger in the quaternary system than in the ternary systems. Brunetti et al. (1989) reported a similar behavior in the much more complex 65% triolein mixture where solubility values were larger than those for triolein of higher purity. Third, although the solubility of a component in a mixture can be very different from its solubility as a pure substance, it is usually the heavier compounds that are affected. The lighter component may act as a co-solvent, increasing the overall solubility of the mixture (Goncalves et al., 1991). Fourth, an effective co-solvent can also be a component, which is somewhat more polar than the supercritical solvent and is a good solvent in its liquid state for the target solute (Hawthorne, 1990). Therefore, canola oil, which is a solvent in its liquid state for carotenes, may act as a co-solvent to enhance the solubility of β–carotene in SC-CO_2.

One of the major components of canola oil, OOO, is highly soluble in SC-CO_2 (Table 1.10) compared to β-carotene (Table 1.11). Not only OOO, but triglycerides of other species are also more soluble in SC-CO_2 than β–carotene in the mixed system.

Table 1.10. Literature solubility data for triolein and trilinolein in SC-CO_2

Component	Temperature (K)	Pressure (MPa)	Solubility range as reported	Solubility (g/g CO_2)	References
Triolein	313-353	8.0-25.0	0.01-71.86 g/L	$0.032\text{-}0.146\times10^{-3}$	Chrastil (1982)
	313, 333	20, 30	0.4-2.2 g/g	0.4-2.2	Brunette et al. (1989)
	323, 333	17.2-30.9	0.06-0.76 wt%	$6\text{-}76\times10^{-4}$	Nilsson et al. (1991)
	308	9.6-22.0	1.1-7.7 mg/nL	$5.6\text{-}38.9\times10^{-4}$	Goncalves et al. (1991)
	313	17.2-31.0	$2.1\text{-}7.0$ w/w 10^{3}	$2.1\text{-}7.0\times10^{-3}$	Nilsson and Hudson (1993)
	308	8.0-21.0	0.077-0.241 g/g	$7.7\text{-}24.1\times10^{-2}$	Ribeiro and Bernardo-Gil (1995)
Trilinolein	313, 333	8.0-25.0	0.04-7.35 g/L	$0.127\text{-}10\times10^{-3}$	Chrastil (1982)

Table 1.11. Literature solubility data for β-carotene in SC-CO_2

Temperature (K)	Pressure (MPa)	Solubility range as reported ($y \times 10^8$)	Solubility (g/g CO_2)	References
313-343	20-50	0.9-254	$0.11\text{-}30.97 \times 10^{-6}$	Cygnarowicz (1990b)
308-323	9.87-29.8	0.085-5.3 g/m^3	$0.00132\text{-}6 \times 10^{-6}$	Sakakl (1992)
288-328	5-50	1.75-130	$2.13\text{-}160 \times 10^{-5}$	Jay and Steytler (1992)
298-313	10-30	0.6-5.5 g/m^3	$0.092\text{-}6 \times 10^{-6}$	Skerget (1995)
310-340	9.73-26	1.51-170	$0.18\text{-}20.73 \times 10^{-6}$	Suba (1997)
313-333	12-27.4	0.2-62.7	$0.024\text{-}7.64 \times 10^{-6}$	Mendes (1999)

When oil was extracted with SC-CO_2 by Franca et al. (1997) from pressed palm oil fibre, it was observed that the amount of carotenes expressed as β–carotene increased with extraction time whereas the amount of triglycerides decreased.

The solubility data for mixed triglycerides are very limited and contradictory. According to Nilsson and Hudson (1993), the two mixed triglycerides species, palmitin-diolein (POO) and olein-dipalmitin (PPO), had higher partition coefficients than OOO, with PPO having the highest. However, the findings of Goncalves et al. (1991) indicated that POO would have a lower solubility in SC-CO_2 than OOO.

Due to the lack of solubility data for the other main components of canola oil (LOO, LnOO, LLO, and LnLO), any further quantitative explanation cannot be given for these mixed pure triglycerides. However, the solubility of canola oil in SC-CO_2 has been studied. At low pressure (20 MPa and 30 MPa), the oil solubility was found to decrease with temperature, whereas at high pressure (36 MPa) the solubility exhibited a maximum of 11 mg/g CO_2 at 55°C (Lee et al. 1986). Temelli (1992) used relatively higher pressures (34.5-62.0 MPa) and reported that the solubility increased as temperature increased at each pressure level, with a maximum solubility of 43.3 mg/g at 70 °C and 62.0 MPa. According to Temelli (1992), canola oil is a multicomponent mixture of nonvolatile triglycerides and their very low vapor pressures did not contribute to solubility significantly at the temperatures studied.

As the co-solvent added into the system may also have some effect on the matrix by swelling the matrix and competing for the adsorption sites, the anticipated solubility enhancement for carotenoids with the addition of canola oil may be caused by the complex interactions among the carrot matrix, carotenoids, canola oil and SC-CO_2.

1.4.4.3. Moisture

According to Hopper and King (1991), water present in biological samples can interfere with the effectiveness of supercritical fluid extraction with CO_2. Polar solutes may be removed more easily from a matrix with a higher moisture content. It is believed that the solute is solubilized in the entrained water and removed from the matrix with the physical removal of water. For nonpolar analytes, the presence of moisture in the matrix can be detrimental. Weathers et al. (1999) demonstrated that water did reduce the extraction efficiency of β–carotene from paprika by a factor of approximately 2. In general, the removal of water from vegetable material prior to extraction facilitates supercritical fluid extraction, because water can form ice in the restrictor, end up in the trap itself, or cause problems if the solute has more affinity for it than for CO_2. However, according to Marsili and Callahan (1993), as a co-solvent in the SC-CO_2 extraction of crystalline β-carotene, water neither significantly increased nor decreased the solubility. This does not account, however, for the matrix effect of water. Moisture content determines the surface structure and activity of materials (Stahl et al., 1988). Solubility of gases in the cell plasma and the elasticity of cell membranes are affected by moisture content, since water has some effect on increasing the permeability of the cell membrane of low moisture content materials through its swelling effect, hence improving accessibility of the oil (Dunford and Temelli, 1997). Goto (1987) extracted carotenoids completely from raw carrots and the extracted residue was colorless, while only about 70% of the carotenoids were extracted from freeze-dried carrots and the residue still had color. Water co-extraction was observed (Snyder et al., 1984; Chao et al., 1991) and was found to be independent of pressure and feed composition, while Dunford and Temelli

(1997) reported an increase with temperature and moisture content of feed material and a decrease with pressure.

1.4.4.4. Particle size

In general, the smaller the particle size of the substrate, the more rapid and complete is the extraction, because of the shorter internal diffusion path lengths over which the solutes must travel to reach the bulk fluid phase. Subra et al. (1998) used three groups of ground carrot samples of different particle size ($x < 0.25$ mm, $0.25 < x < 0.4$ mm, $0.4 < x < 1$ mm) and concluded that the smallest size led to a higher extraction yield, which indicated that the grinding liberated more carotenoids by destroying the inner structures of the particles. An increasing amount of extract versus particle size was also reported by Goto et al. (1987), who conducted extractions with freeze-dried carrots of particle sizes with a diameter of 0.26, 0.47 and 1.12 mm. They concluded that only the carotenoids present in about 10 µm of the outer shell of each particle, which corresponds approximately to the size of a broken carrot cell, could be extracted rapidly and that the cellular structures should be broken to get a complete extraction of substrate. Tehrani (1993) suggested a sample size of 1-2 mm for complete extraction; however, Tena et al. (1994) suggested sizes down to 0.01–0.1 mm. Baysal et al. (2000) used particles of 3 mm from tomato paste waste and did not recommend milling the sample to particles < 2 mm, as such particles would block the steel mesh filters and piping between the extractor and separator. In his review, Taylor (1995) stated that particles smaller than 0.05 mm should be avoided because they may become compacted in the vessel, which can lead to channeling of the SCF and inefficient contact between the SCF and the matrix, as well as the particles may be mechanically forced out of the vessel during pressurization.

1.4.4.5. Flow rate

Flow rate is another important factor affecting the extraction yield and speed, and greatly interfere with particle size. Solvent flow rate exerts its effect on the extraction mostly in the last two steps described in Section 1.4.3. If the substrate has larger particle size, it will take a longer time for the extractable material to be transferred to the bulk fluid, and thus the third step becomes rate-limiting, A slower dynamic flow rate may enhance overall recovery by increasing the time the solute has to reach equilibrium in the extraction phase. If it is quite easy for the extraction solvent to dissolve the extractable material and quickly transfer to the bulk fluid due to the small particle size, then the fourth step may become rate-limiting, and reducing the time to remove the extraction solution from the extraction vessel should enhance overall recovery. This can be accomplished by increasing the dynamic flow rate.

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2. EFFECT OF EXTRACTION PARAMETERS ON THE SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF CAROTENOIDS FROM CARROT[1]

2.1. INTRODUCTION

Carrot is the second most popular vegetable in the world after potatoes (Anonymous, 2003). In Canada, excluding potatoes, carrot is the top vegetable in the fresh market and in third place in processed vegetables (Table 2.1). In the U.S., the carrot production in 1997 was 34.3% higher than that in 1987. Another 18.7% increase is expected by the year 2007 (Brandenberger, 1999). This increase in carrot production is being encouraged by an increase in carrot consumption. Among the major nutrients that carrots provide (Table 2.2), carotenoids, the main pigments that are responsible for the color of carrots, are probably of the most importance to food and nutrition scientists. According to Chen et al. (1995), β-carotene constitutes a large portion (60-80%) of the carotenoids in carrots, followed by α-carotene (10-40%), lutein (1-5%) and the other minor carotenoids (0.1-1%). The carotene content varies significantly among phloem, peel and xylem, which are the three tissues of carrot (Table 2.3).

Carotenoids have been used as natural food colorants. These colorants not only meet the consumers' demand for "natural" products, but also provide the consumers some nutritional functions such as provitamin A and antioxidant activity. Vitamin A deficiency is not a problem in the developed countries due to the high consumption level

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[1] Aversion of this chapter is to be submitted to the *J. Supercrit. Fluids* for consideration for publication.

Table 2.1. Major vegetable production in Canada[1]

Product	1995	1996	1997	1998	1999
Processed vegetables (tonnes)					
Tomatoes	462,882	457,791	450,934	509,783	495,085
Corn	226,937	264,017	285,312	252,551	228,461
Carrots	60,070	75,404	82,427	79,279	74,366
Peas	50,126	64,265	69,824	65,605	69,245
Cucumbers	38,166	39,703	41,585	47,936	55,604
Beans	33,894	34,245	35,109	42,919	42,824
Total	908,838	1,002,878	1,029,066	1,047,422	1,028,343
Fresh market vegetables (tonnes)					
Carrots	207,955	246,383	210,129	235,376	219,858
Onions, dry	156,410	158,872	150,860	152,570	162,939
Cabbage	139,423	139,466	121,814	134,925	118,748
Lettuce	59,994	92,366	95,805	89,823	83,627
Corn	97,956	90,408	72,139	94,474	70,600
Total	1,058,048	1,149,843	1,015,526	992,026	972,696

[1] Adopted from Anonymous (2001b)

Table 2.2. Major nutritional value of fresh carrots

Nutrient	Value per 100 g of edible portion		
	Ref.[1]	Ref.[2]	Ref.[3]
Proximate analysis (g/100 g)			
Water	87.79	88.8	86.0
Protein	1.03	0.7	0.9
Lipids	0.19	0.5	0.2
Carbonhydrate	10.14	6.0	10.6
Fiber, total dietary	3.00	2.4	1.2
Ash	0.87	----	1.1
Minerals (mg/100 g)			
Calcium, Ca	27	34	80
Iron, Fe	0.50	0.4	1.03
Magnesium, Mg	15	9.0	----
Phosphorus, P	44	25	530
Potassium, K	323	240	----
Sodium, Na	35	40	----
Vitamins (mg/100 g)			
Carotenes	----	5.33	----
Vitamin A, RE	2.81	----	----
Niacin	0.93	0.2	----
Vitamin C, total ascorbic acid	9.30	4.0	----
Vitamin E	0.46	----	----

[1] Adopted from Anonymous (2001a)
[2] Adopted from Holland et al. (1991)
[3] Adopted from Gopalan et al. (1991)

Table 2.3. Distribution of carotenes in fresh carrot tissue[1]

Tissue	α-Carotene	β-Carotene	Total carotenes	α-Carotene	β-Carotene	Total carotenes
	ppm (fresh wt.)			ppm (dry wt.)		
Phloem	70.6	101.9	172.4	581.5	841.4	1423
Peel	58.9	88.6	147.5	489.6	725.5	1205.2
Xylem	26.5	43.1	69.6	234.5	380.7	615.2

[1] Adopted from Howard and Dewi (1996)

of carrots along with other animal-based food products. For example, in the U.S., carrots contribute 30% of the total vitamin A consumption (Simon, 2003). In contrast, vitamin A deficiency remains a serious public health problem in developing countries. Dietary sources and inadequacy of provitamin A intake continue to be a major concern in these countries. On the other hand, the focus of carotenoids in the developed world has shifted to their other health-promoting effects. Owing to their antioxidant activity, carotenoids are efficient in scavenging free radicals, which are thought to play a role in the pathophysiology of various chronic diseases in the developed countries. Although there is yet no definitive proof, some evidence has been presented that they have important effects in cancer prevention (Shekelle et al., 1981; Krinsky, 1988), reduction of the risk of heart disease (Kardinaal et al., 1993) and age-related macular degeneration and cataract (Seddon et al., 1994; Hammond et al., 1997).

In agriculture, however, each year, a notable amount of fruits and vegetables becomes waste after harvest due to defects in size or shape and defects caused by diseases, insects or wounds. Also, a significant amount of agricultural products are left in the field, for example as culled carrots or potatoes. These factors result in reduced economic return to the producers. In the food processing industry, a similar problem occurs due to the processing waste. Taking a cut-and-peel baby carrot processing as an example, approximately 60% of fresh carrots become waste after the processing (Lazcano et al., 1998). The usage of these portions of fruits and vegetables has been limited to juice processing or livestock feed. In some cases, it can even become a financial liability and environmental problem, as some food processing and farm wastes that have no food or feed uses are difficult to dispose of properly. Hence, besides minimizing the residual waste originating from the farm and food processing, exploring the potential of recovering high-value components in the waste and incorporating them into food products is another optimal strategy to enhance the overall economic profile. In the case of carrots, extraction of the "natural" high-value pigment, carotenoids, which has great potential as a nutritional supplement, seems to be an effective strategy.

In the past few decades, carotenoids have been mainly extracted with organic solvents (O'day et al., 1982; Aravantinos-Zafriris et al., 1992; Zelkha, et al., 1997; Todd, et al., 1998). Other than the direct cost of the solvent, the disposal and/or recovery cost of the solvent waste, and the high cost of removal of the potentially toxic solvent residues from the product, this extraction method can result in the degradation of a considerable amount of active components. Besides, the usage of organic solvents in industrial scale is restricted by government regulations due to growing consumer concerns regarding solvent residues in the final product as well as their environmental impact. On the contrary, supercritical fluid extraction (SFE), particularly, supercritical carbon dioxide (SC-CO_2) extraction was found to be an effective alternative and has gained increasing interest.

Structurally, β-carotene is an unsaturated hydrocarbon chain with cyclohexene rings at both ends. The lack of strong polar moieties in the molecular structure would suggest that the solubility of β-carotene in SC-CO_2 should be fairly high. However, despite its relatively non-polar structure, the molecular weight is large (536.85), a factor known to inhibit solubility in SC-CO_2 (Dandge et al., 1985) due to low volatility caused by the large molecular weight. The addition of a co-solvent to SC-CO_2 was proven to improve the efficiency (Goto et al., 1987; Barth et al., 1995; Vega et al., 1996; Jaren-Galan et al., 1999; Baysal et al., 2000; Cadoni et al., 2000; Naranjo-Modad et al., 2000). However, the co-solvent that has been employed in most of these studies is ethanol, which is a polar solvent and is not the best choice for improving the solubility of the nonpolar carotenoids in SC-CO_2. Besides, ethanol has to be removed from the final product. Other co-solvents that were studied are organic solvents, which negate the major advantage of supercritical fluid technology of being able to produce "natural" extracts.

On the other hand, based on the following experiments and findings, it seems worthwhile to explore other co-solvents for carotenoid extraction with SC-CO_2. First, Peter and Brunner (1980) showed that the relative volatilities of components can be altered by the addition of a co-solvent and the solubility of the nonvolatile components in compressed gas increased with the concentration of co-solvent. Second, Bamberger et al. (1988) reported that the solubility of a less volatile lipid component was significantly enhanced by the presence of a more volatile triglyceride species in the system. Third, Goncalves et al. (1991) demonstrated that in a multicomponent system of SC-CO_2 and at least two chemical components, the solubility of the heavier compounds were affected by the lighter components. Fourth, Hawthorne (1990) stated that an effective entrainer can be a component, which is somewhat more polar than the supercritical solvent and is a good solvent in its liquid state for the target analyte. Canola oil, with its major component triolein (OOO), is highly soluble in SC-CO_2 when compared to carotene and is a good solvent for carotenes in its liquid state. Therefore, canola oil may act as a co-solvent to adjust the polarity of the solvent mixture and the volatility of carotenes, making CO_2 more powerful to dissolve the solutes and thus enhance the solubility of β–carotene.

The main objective of this study was to investigate the potential of using canola oil as a co-solvent for the SC-CO_2 extraction of carotenoids from carrots. The specific objectives were:

1. to study the effect of various extraction parameters, such as canola oil concentration, pressure, temperature, CO_2 flow rate, particle size and moisture content of carrots on the carotenoids extraction, and
2. to determine the composition of the carotenoid extracts obtained under different conditions.

2.2. MATERIALS AND METHODS

2.2.1. Materials

Carrots (*Apache* variety) were provided by I & S Food Services Ltd., Edmonton, AB. These carrots were harvested by Grimmway Farm in Imperial Valley, CA in early May, 2002. Carrots were transported in open bed trailers at ambient temperature. Once processed, they were stored at 1-3 °C in Edmonton.

Carbon dioxide and nitrogen were 99.95% (w/w) purity, bone dry and obtained from Praxair Canada Inc. (Mississauga, ON). Canola oil (No Name, 100% pure) was bought from a local supermarket. β-Carotene (≥ 97% UV purity) and lutein (~ 70% UV purity), which were from Sigma Chemical Co. (St. Louis, MO), were used as standards in high-performance liquid chromatography (HPLC) analysis. Hexane, acetone, methanol, dichloromethane, and acetonitrile were all HPLC grade and were obtained from Fisher Scientific (Fair lawn, NJ) along with petroleum ether and anhydrous sodium sulphate.

2.2.2. Carrot sample preparation

Upon receipt of carrots (25 kg), they were washed thoroughly with cold tap water, wiped with tissue paper, and left on the bench for a few minutes for drying of surface moisture. The carrots were then chopped into 5 mm cubes at room temperature with a food chopper (Model 84185, Hobart Corporation, Troy, OH). The carrot cubes were well mixed. A small portion of carrot cubes were retained for proximate composition analysis. The remaining portion was vacuum packed in several moisture and O_2-barrier bags (3 MIL, 75% polyethylene and 25% nylon, Unipack, Edmonton, AB) as thin layers and then frozen overnight at -30 °C. On the following morning, the frozen carrot cubes were placed on stainless steel trays and freeze-dried (Model FFD-42-WS, Virtis Co. Inc., Gardiner, NY) for 5 days until moisture content was approximately 0.8%. The desired particle size distribution (0.25-0.5 mm, 0.5-1 mm and 1-2 mm) was achieved by grinding the cubes with a Quadro Comil grinder (Model 197S, Emerson Industrial Controls, Grand Island, NY) and sieving (W.S. Tyler Company of Canada Ltd., St. Catharines, ON) prior to extraction. All the ground samples were vacuum packed in several moisture and O_2-barrier bags, labelled and stored at –18 °C in dark to minimize any carotenoid degradation.

Moisture content of carrot samples was modified by adding milli-Q water to dried carrot sample of particle size 0.25–0.5 mm, followed by overnight tempering at 4 °C in the dark prior to extraction. The actual moisture content of the samples prior to extraction (84.6%, 48.7%, and 17.5%) was determined according to the methodology given below.

2.2.3. Proximate composition analysis

Each proximate analysis described below was carried out in triplicate.

2.2.3.1. Moisture

Approximately 5 g fresh carrot cubes or 2 g freeze-dried carrot sample were weighed into aluminum dishes (50 mm diameter and 23 mm deep). With covers completely open, dishes and covers were placed in the vacuum oven (Model 5851, National Appliance Co., Portland, OR) and dried under vacuum (1.0×10^4 Pa) at 70 °C for 24 h. The lids were then closed and the dishes were transferred to a desiccator to cool and weighed. The moisture content was calculated on wet basis as

$$\%\ (\text{w/w})\ \text{moisture} = 100 \times (\text{wt. of loss on drying, g / wt. of sample, g}) \qquad (2.1)$$

2.2.3.2. Ash

Ash content was determined according to Official Method 942.05 (AOAC, 2000). Approximately 2 g fresh carrot cubes were weighed into a porcelain crucible and placed in a muffle furnace (Model F-A1730, Thermolyne Corp., Dubuque, IA) set at 525 °C overnight. The crucible was transferred to a desiccator, cooled and weighed.

$$\%\ (\text{w/w})\ \text{ash} = (\text{wt. of residue ash, g / wt. of test portion, g}) \times 100 \qquad (2.2)$$

2.2.3.3. Protein

Nitrogen content was determined using a nitrogen analyzer (Model fp-428, Leco Instruments Ltd., Mississauga, ON). Protein content was estimated by using a conversion factor of 6.25 (Temelli, 1997).

2.2.3.4. Lipid

Crude lipid content was determined using a Goldfisch Extraction Unit (Labconco, Kansas City, MI). Approximately 2 g sample were weighed into a cellulose extraction thimble (25 mm I.D. x 80 mm E.L., Whatman International Ltd., Maidstone, England) and covered with a small amount of glass wool (Supelco, Inc., Bellefonte, PA). The extraction beakers were weighed and approximately 40 mL petroleum ether was put into the beaker. One blank, which carried only 40 mL petroleum ether, was prepared and run through the entire extraction process. After attaching the sample holders and the extraction beakers to the Goldfisch unit, the system was heated with the condenser water on and the extraction was continued for 5 h. At the end of the extraction period, petroleum ether was evaporated from beakers and the beakers were placed in an 110 °C oven (Despatch Oven Co., Minnneapolis, MN) for 30 min to remove any residual moisture. The beakers were then cooled to room temperature in a desiccator and weighed. The crude lipid content was calculated on dry basis as follows:

$$\% \text{ Lipid}=100\times(\text{wt. extract - wt. blank residue}) / [\text{wt. sample}\times(1-\% \text{ moisture}/100)] \quad (2.3)$$

2.2.3.5. Carbohydrates

Carbohydrate content was calculated based on 100% minus the sum of moisture, ash, protein and lipid contents.

2.2.4. Traditional solvent extraction (TSE)

Traditional solvent extraction of carotenes was conducted according to Official Method 941.15 (AOAC, 2000). Approximately 2 g freeze-dried carrots were homogenized with a Tissumizer (Model SDT 1810, Tekmar, Cincinnati, OH) for 5 min at

70 rpm with 100 mL hexane/acetone (6:4 v/v) containing 0.005% (w/v) butylated hydroxytoluene (BHT). The sample was filtered in vacuo and the residue was washed first with acetone (2 × 25 mL) and then with 25 mL hexane. The combined organic filtrate was washed three times with 100 mL milli-Q water. The organic phase was dried over gentle nitrogen flow. The dried extraction residue was weighed and stored at -18 °C in the dark until analysis.

2.2.5. Supercritical fluid extraction

A Supercritical Fluid Extraction Screening System (Newport Scientific Inc., Jessup, MD) as shown in Figure 2.1 was used in this study. Carrot sample (2 ± 0.1 g) was weighed and loaded into a stainless steel basket (15 cm × 13 mm I.D.), the ends of which were fitted with two 10 μm metallic filters (Mott Metalurgical Co., Farmington, CA) to avoid any sample carry over. Two thin layers of glasswool were also put inside the ends of the basket to avoid sample carry over. The desired extraction temperature was achieved by heating the extraction vessel (300 mL, 300 SS) with two silicon fibreglass electrical resistance type heating tapes (100 W each), and the temperature was monitored by a thermocouple immersed at the centre of the extractor and regulated by a controller. CO_2 was filtered and compressed to desired pressure by a diaphragm compressor. The extraction pressure was controlled by a back pressure regulator (Tescom Co., Elk River, MN). A rupture disc was provided for pressure overshoot protection. The flow rate of CO_2 (measured at ambient conditions) passing through the extractor was controlled by manual adjustment of a metering valve, which was heated to prevent freezing upon depressurization of SC-CO_2. Canola oil was introduced into the extraction system as the

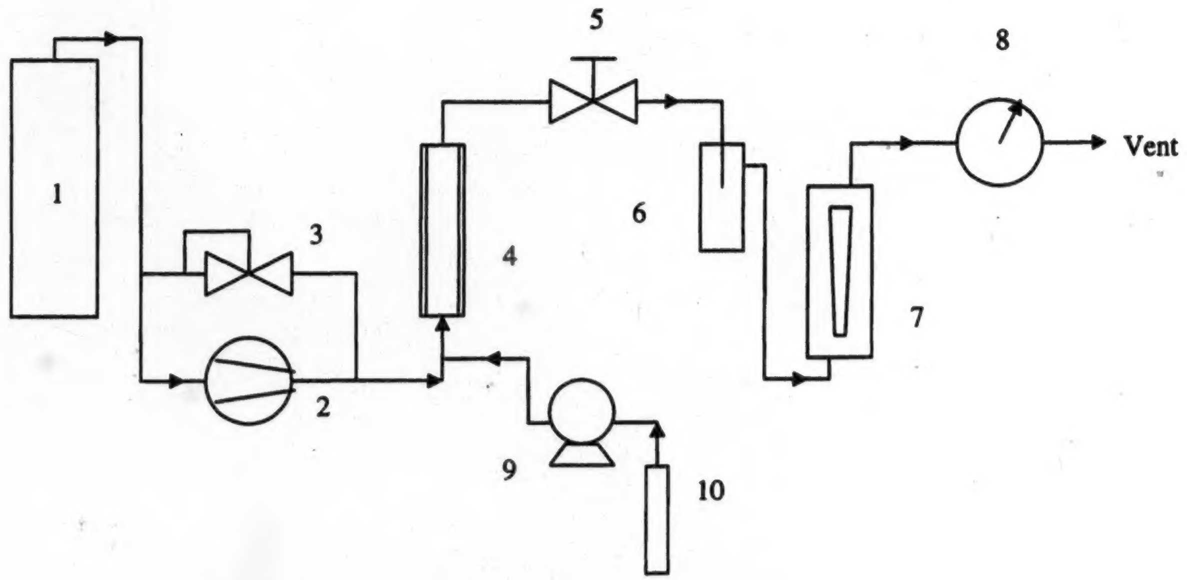

Figure 2.1. Supercritical fluid extraction screening system

1. CO_2 Tank, 2. Compressor, 3. Back pressure regulator,
4. Extraction vessel with heating jacket, 5. Depressurization valve,
6. Extract collection tube, 7. Rotameter, 8. Dry gas meter,
9. Piston pump, 10. Canola oil reservoir

co-solvent by setting the flow rate of a piston pump (Gilson 305, Gilson, Inc., Middleton, WI) to achieve the desired oil concentration of 2.5 and 5% (w/w) in SC-CO_2.

The extractions were performed for 4 h and the extracted oil was collected in side-armed glass tubes (wrapped with aluminum foil to avoid light degradation) attached after the depressurization valve, which were held in a refrigerated circulating bath (Lauda, Model RMT-6, Brinkmann, Rexdale, ON) at -20 °C. For the extractions with canola oil addition, the extraction was continued for an additional 3 h to remove the remaining canola oil that was introduced as co-solvent during the original 4 h extraction. Depressurized CO_2 was passed through a rotameter and the volume of CO_2 used was recorded by a dry gas meter (American Meter Co., Horsham, PA) before venting to atmosphere. The extracted oil was washed several times with hexane into a pre-weighed glass vial containing a known amount of BHT. The hexane was then removed under gentle nitrogen flow. The extracted oil samples were weighed and stored at -18 °C in dark until analysis.

To investigate the effects of various extraction conditions, including temperature, pressure, canola oil concentration, particle size, flow rate and moisture content of the feed material, the experimental design consisted of four sections as follows:

1. *The effects of temperature, pressure and canola oil concentration* on SC-CO_2 extraction of carotenoids were tested by a complete 3^3 factorial design. Six repetitions were performed at the central design point to estimate the reproducibility of the experimental results. The three levels of temperature were 40, 55 and 70 °C, whereas those of pressure were 27.6, 41.3 and 55.1 MPa. The three levels of canola oil addition in

$SC\text{-}CO_2$ were 0, 2.5, and 5% (w/w). For ease of operation of the experiments, the dried carrot was used as feed material. Particle size of 0.5-1 mm and flow rate of 1 L/min were chosen because they were the middle point of the levels tested for each parameter as described in later sections. The extracts were collected as one fraction throughout the 4 h extraction and were analyzed using HPLC as described below.

2. *The effect of particle size* on $SC\text{-}CO_2$ extraction of carotenoids was tested using dried carrot sample of different particle sizes (0.25-0.5, 0.5-1, and 1-2 mm). Temperature and pressure were kept constant at 70 °C and 55.1 MPa, respectively. The level of canola oil addition as co-solvent was maintained at 5% (w/w). The flow rate was fixed at 1 L/min. The extracts were collected in 8 fractions every 30 min until 4 h. Extractions for each particle size were carried out in duplicate and analyzed by HPLC.

3. *The effect of moisture content* of the feed material (84.6%, 48.7%, and 17.5%) on the $SC\text{-}CO_2$ extraction of carotenoids was tested at 70 °C, 55.1 MPa, and canola oil concentration of 5% (w/w). The particle size of feed material was maintained at 0.25-0.5 mm and the flow rate was fixed at 1 L/min. Extract fractions were collected every 30 min until 4 h. Extractions for each moisture level were carried out in duplicate and analyzed by HPLC.

The amount of water co-extracted with carotenoids was determined as follows: After each extraction collection tube was removed from the extraction unit, it was left in dark at room temperature for 30 min to remove any residual CO_2. The total weight of the tube with extract was recorded and the total extract weight was calculated. Anhydrous sodium sulphate was added to the extraction tube until the water in the extract was completely absorbed. Then, the oil extract was washed out into a pre-weighed vial with hexane and the weight of oil extract was determined. The weight of the water extract was calculated as the weight of the total extract minus the weight of the oil extract.

4. *The effect of CO_2 flow rate* (0.5, 1, and 2 L/min) on the SC-CO_2 extraction of carotenoids was tested with carrot samples of 0.8% moisture content and 0.25-0.5 mm particle size at 70 °C, 55.1 MPa and canola oil concentration of 5% (w/w). Extract fractions were collected every 30 min until 4 h. Extractions for each flow rate level were carried out in duplicate and analyzed by HPLC.

2.2.6. Carotenoids identification and quantification

Carotenoids identification and quantification were performed by HPLC analysis according to a modified method of Mendes et al. (1999). HPLC analysis of carotenoids was performed using a Shimadzu chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD). A Supelcosil™ LC-18 column, 15 cm x 4.6 cm, 5 µm (Supelco, Inc. Bellefonte, PA) was used to separate individual carotenoids at ambient temperature. Samples (50 µL) in methanol/dichloromethane (1:1 v/v) were injected by a Hewlett Packard 1050 autosampler. Methanol with 10% (v/v) acetonitrile was used as mobile phase. The mobile phase was delivered at a flow rate of 1 mL/min under isocratic conditions by Varian 9010 solvent delivery system (Varian Associates, Sugar Land, TX). The separated lutein, α-carotene and β-carotene peaks were monitored by a UV detector (Model Spectromonitor II, Laboratory Data Control, Riviera Beach, FL) operated at 450 nm wavelength. β-Carotene and lutein standards were used for identification and quantification based on a calibration curve. Since it was not possible to obtain α-carotene standard, it was assumed that α-carotene has a similar response factor to that of β-carotene and the concentration of α-carotene in various samples were calculated based on the calibration curve of β-carotene. A typical HPLC chromatogram of a SC-CO_2 extract is shown in Figure 2.2.

2.2.7. Statistical analysis

The effects of temperature, pressure, and canola oil concentration on the carotenoid extraction yield were analyzed by STATGRAPHICS Plus Version 5 (Manugistics Inc., 2000). Analysis of variance of carotenoid yield was performed to test the main three effects (temperature, pressure and canola oil concentration) and the two way interaction effects between them. The multiple regression equation, the estimated response surface plot, and the optimum combination of extraction factors were reported for the carotenoid yield. The effects of particle size, flow rate and moisture content of feed material on carotenoid yield were analyzed by the General Linear Model procedure of SAS Statistical Software, Version 8 (SAS Institute Inc., 1999).

2.3. RESULTS AND DISCUSSION

2.3.1. Proximate composition

The proximate composition of fresh carrot was presented in Table 2.4. The values of moisture, ash, protein and lipids were all in the ranges of data reported by others as summarized in Table 2.2 (Holland et al. 1991; Gopalan et al. 1991; Anonymous, 2001a). The carbohydrate content was comparable but slightly higher than those reported in Table 2.2. The carotene content was reported by Holland et al. (1991) as 5.33 mg/g (Table 2.2),

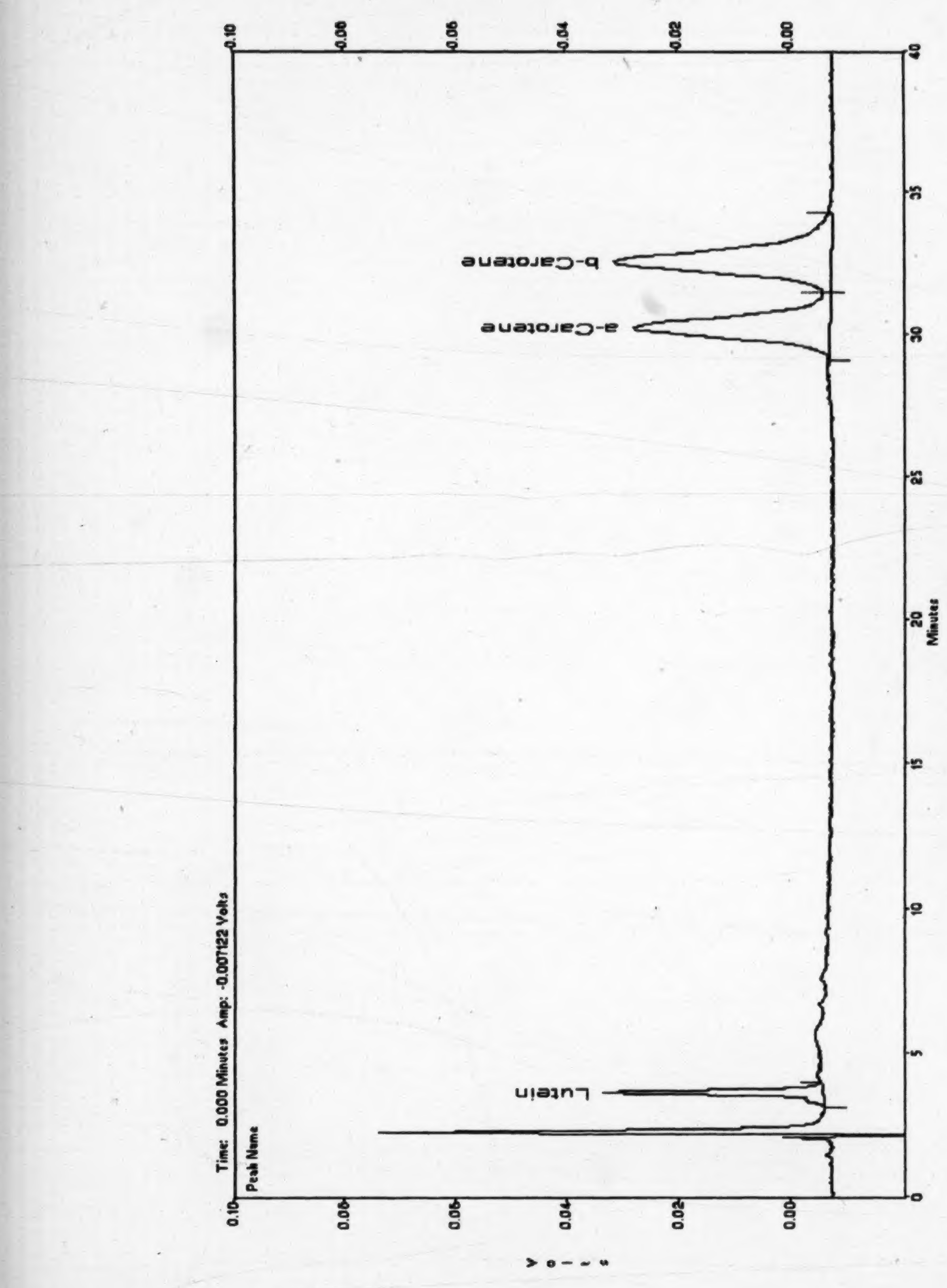

Figure 2.2. Typical chromatogram for the carrot extract

Table 2.4. Proximate composition and carotenoid content of fresh carrots[1]

Proximate (g/100 g fresh wt.)	
Moisture	86.77±0.03
Ash	0.88±0.03
Protein	1.00±0.04
Lipid	0.27±0.002
Carbohydrate	11.08
Carotenoids (TSE, µg/100g fresh wt.)	
α-Carotene	6940±980
β-Carotene	8080±1310
Total carotene	15020±2290

[1] Means ± standard deviation based on triplicate determinations.

but they did not clearly specify the break down of individual carotenes, so no comparison could be made with the results of this study. However, Bajaj et al. (1980) reported the β-carotene content of fresh carrots as 850-8,500 μg/100 g and the result of this study (8,080 μg/100 g of fresh carrot) was within their upper limit. Simon and Wolff (1987) studied six typical orange carrots, representing a diverse range of fresh market carrot and one dark orange selection from the U.S. Department of Agriculture carrot improvement program grown over three years and three locations in the U.S. They reported that the sum of carotenes (β-, α- and ζ-carotene) was in the range 6,300-21,300 μg/100 g of fresh carrot for the six typical carrots and 25,100-54,800 μg/100 g for the dark orange carrot. The predominant carotenoid in all samples was β-carotene, which represented 44-79% of all carotenes quantified. The sum of α- and β-carotene content of the carrot used in this study was 15,020 μg/100 g, which was in the middle range of the total carotenoids content of the six typical carrots. Heinonen (1990) studied 19 cultivars of orange carrots grown in Finland and reported that the predominant carotenoids, α- and β-carotene, ranged between 2,200-4,900 and 4,600-10,300 μg/100g of fresh weight, respectively. γ-Carotene and lutein were also identified in those 19 cultivars and ranged between 630-2,700 and 110-560 μg/100 g, respectively. The carotene contents of four varieties of raw carrots grown at six locations were also reported by Skrede et al. (1997), with α- and β-carotene contents, ranging from 163 to 346 and 439 to 770 μg/g dry carrots, respectively. Compared with these two studies, the α-carotene content of the carrot in this study was much higher, with a 6,940 μg/100 g of fresh and 427 μg/g of dry carrot. The different carotenoid contents reported in all these studies might be contributed by the differences in carrot genotype, development stage and growing conditions such as temperature and use of fertilizers (Heinonen, 1990).

2.3.2. Effects of temperature, pressure, and canola oil concentration

2.3.2.1. Total oil

To test the reproducibility of the extraction unit, six runs were carried out with 0.5-1 mm dried carrot particles at 55 °C, 55.1 MPa, 2.5% of canola oil concentration and 2 L/min CO_2 flow rate. The coefficient of variation for the total oil amount obtained from these six runs was 5.6%. The yield of crude carrot oil extracted with TSE and SFE (SC-CO_2 without canola oil addition) under different combinations of temperature and pressure was presented in Figure 2.3. The crude oil yields of SFE were 1.83-2.69 g/100 g dry carrot, which were similar to that obtained with TSE (2.46 g/100 g dry carrot). Theoretically, the solubility of carotenoids in supercritical CO_2 increases with pressure at constant temperature. Because of the cross-over of solubility isotherms, in general, the solubility decreases with temperature at pressures below the cross-over pressure and increases with temperature at higher pressures. However, the values reported in Figure 2.3 were the total amount of extract collected after 4 h, which may not correspond to solubility depending on the shape of the extraction curve, since it may have already reached the diffusion-controlled region.

Compared with the amount of canola oil introduced into the system as a co-solvent (at CO_2 flow rate of 1 L/min, ca. 12 g canola oil was pumped into the system at 2.5% oil addition and ca. 24 g at 5% oil addition), the amount of crude oil in 2 g dry carrot is extremely small (approximately 0.04 g). Therefore, the total amount of oil

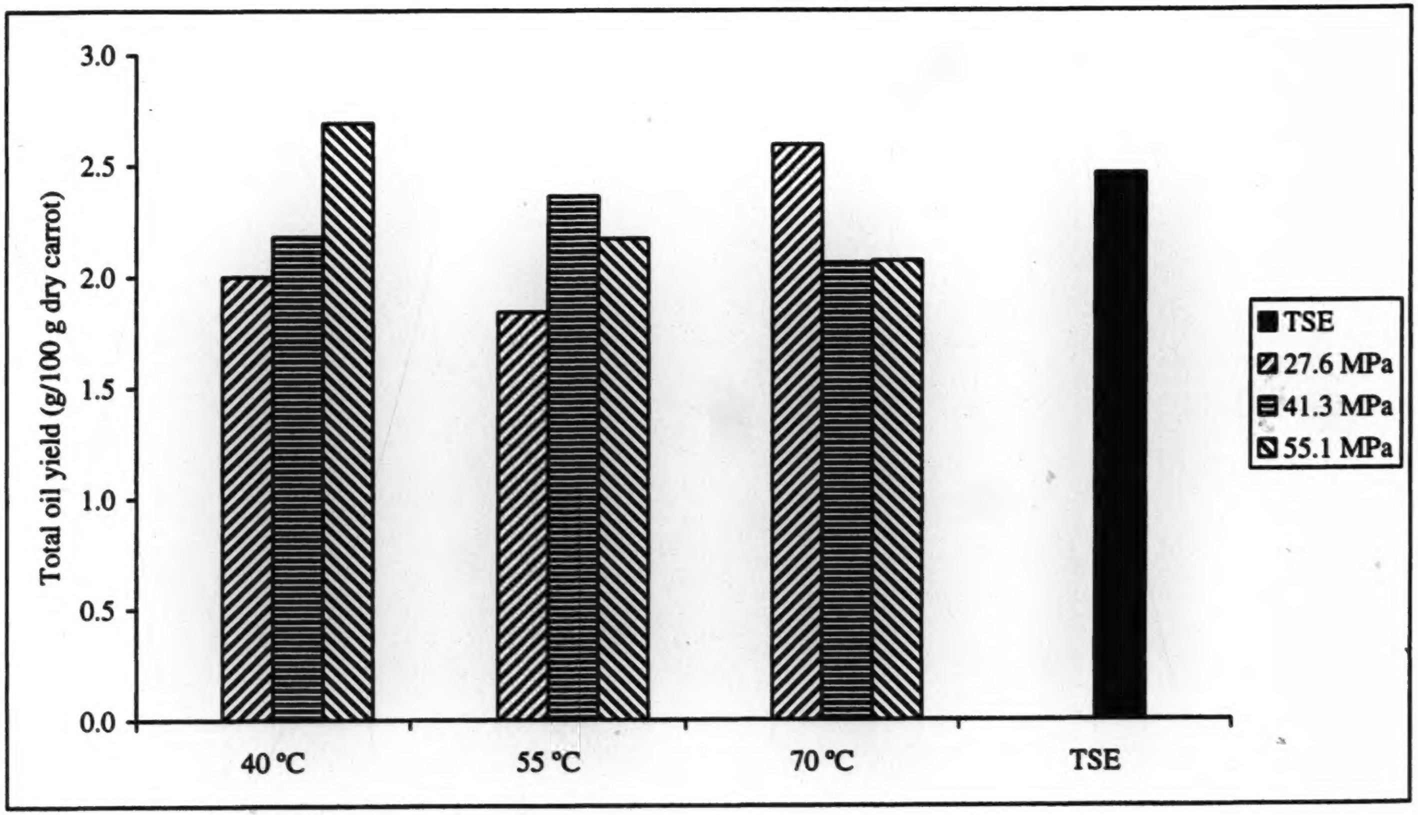

Figure 2.3. Extraction yield of crude carrot oil with traditional solvent extraction (TSE) and supercritical fluid extraction (SFE) without co-solvent addition

collected after extraction with SC-CO_2 with canola oil addition was mainly the canola oil added to the system as a co-solvent, plus a very small amount of crude carrot oil extracted from the feed material. However, it was not possible to separate the crude carrot oil from the canola oil added as co-solvent. The total amount of oil collected after the 4 h SC-CO_2 extraction with canola oil addition at 2.5% level was 2.39 to 7.51 g, which was 43 to 153 times higher than that obtained from the extraction without oil addition at the corresponding temperature and pressure conditions, while the total amount of oil collected after the 4 h extraction with canola oil addition at 5% level was 1.46 to 9.69 g, which was 29 to 217 times higher than that obtained from the extraction without oil addition. The oil remaining in the extraction vessel after the 4 h extraction was removed by an additional 3 h extraction with SC-CO_2 alone. The total amount of oil collected in 4 h increased with pressure at both levels of canola oil addition and the increase became more substantial at higher temperatures and higher level of canola oil addition (Fig. 2.4).

2.3.2.2. Carotenoids yield

The carotenoid yields obtained from the SC-CO_2 extraction at all combinations of temperature, pressure and canola oil concentration and the carotenoids content of the starting material were shown in Figure 2.5. According to one canola oil manufacturer (Canbra Foods Ltd., Lethbrige, AB), no carotenoids were added into the canola oil during processing and carotenoids are not natural constituents of canola oil (Przybylski, 2001). In the mean time, as precautions were carefully taken to minimize carotenoids degradation due to sample handling by covering the extract collection tubes with aluminum foil, keeping the extracts in cold bath (-20 °C) throughout extraction, keeping

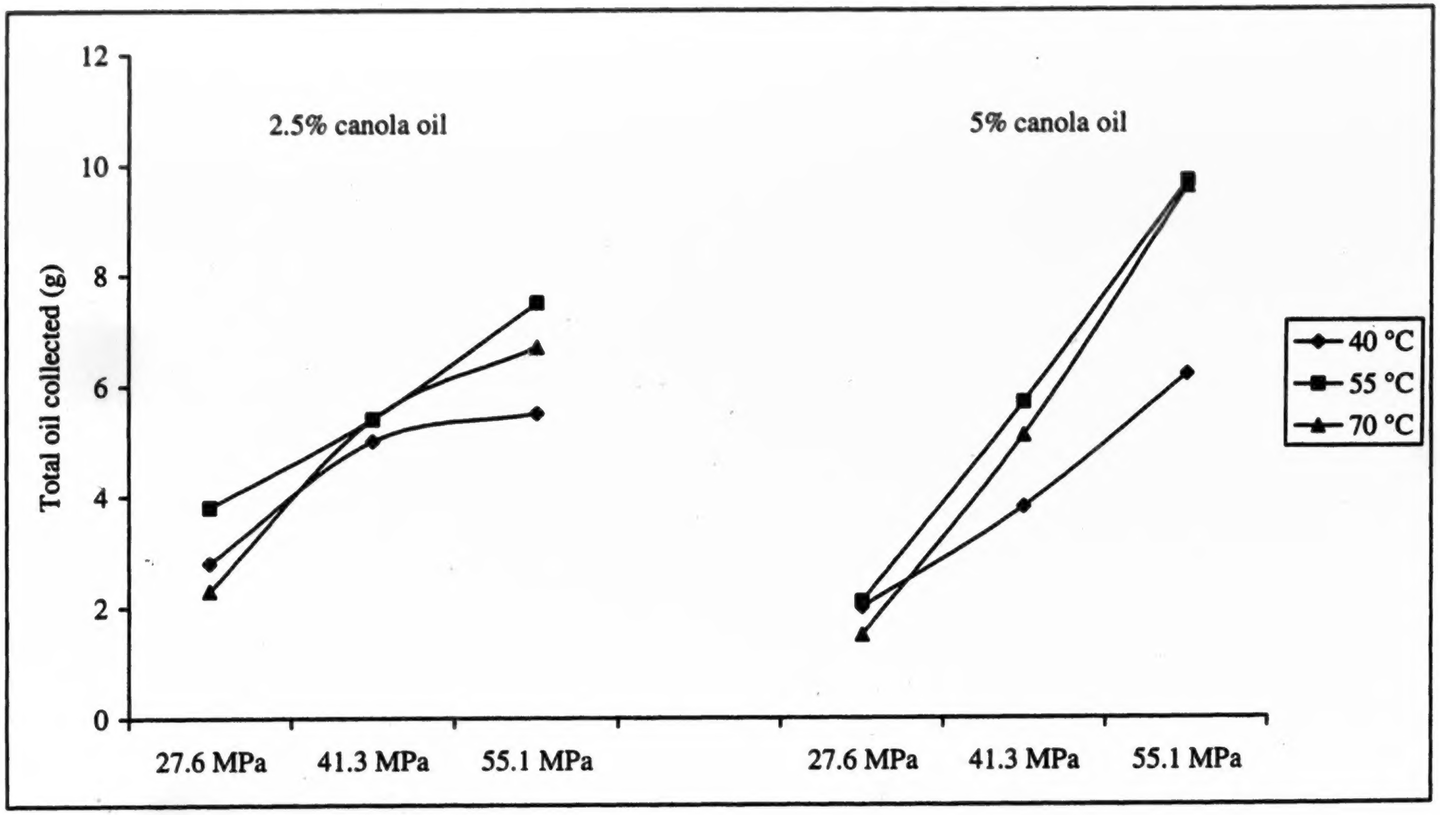

Figure 2.4. Total amount of oil collected in 4 h with SC-CO_2 with canola oil addition as a co-solvent.

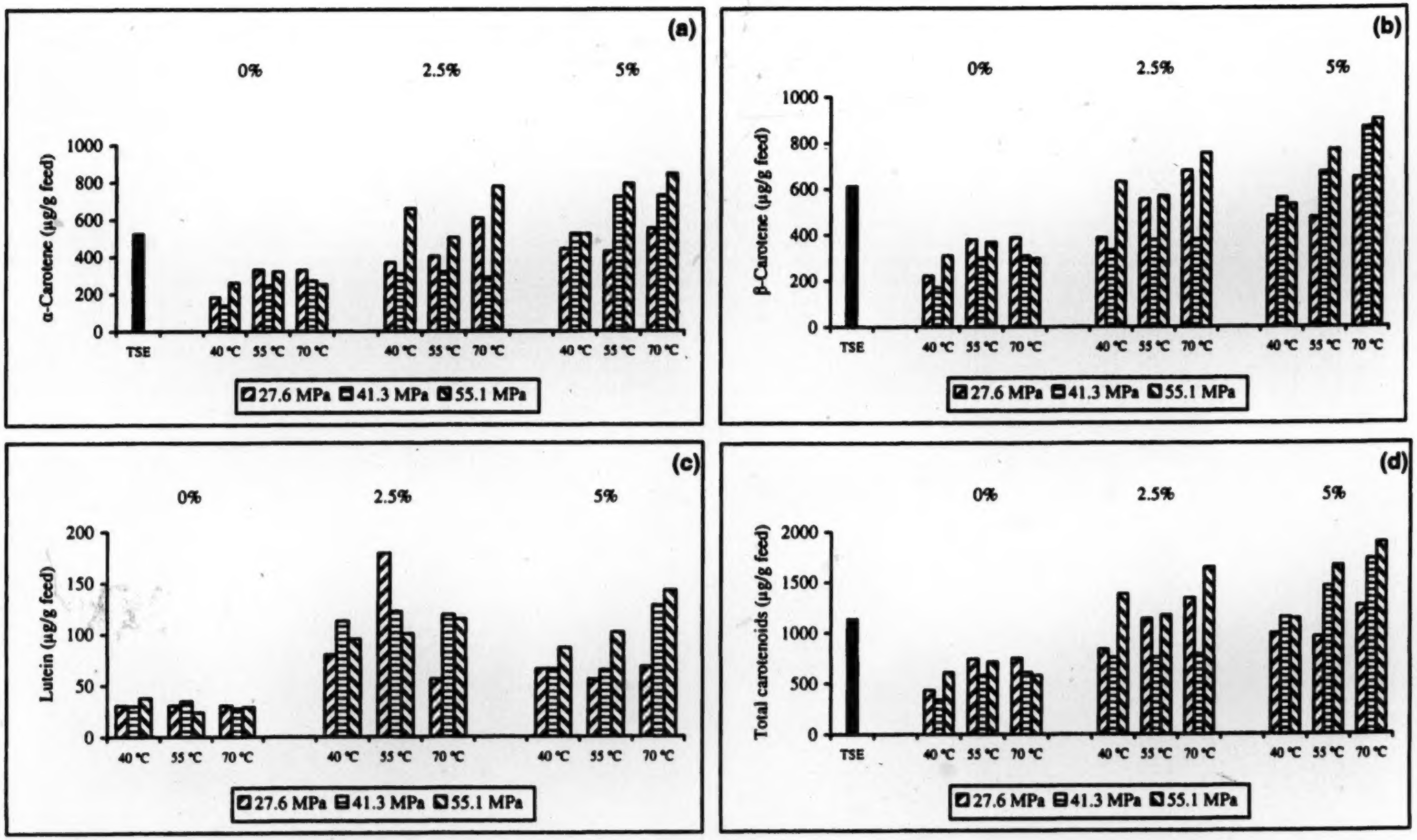

Figure 2.5. Carotenoids yield (µg/g feed, dry matter basis) obtained from TSE and SFE (4 h extraction) at all combinations of temperature, pressure and canola oil concentration studied, (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids

the extracts in a freezer (-18 °C) following extraction and adding BHT to extracts, the results obtained based on the HPLC analysis were assumed to represent the true amount of carotenoids extracted from the carrot sample. Any potential oxidation of carotenoids throughout extraction is avoided in the CO_2 environment. In addition, the vials containing the carotenoid extracts were flushed with nitrogen prior to storage at -18 °C. The metering valve was heated with a heating tape just enough to prevent freezing due to expansion of CO_2 during depressurization and the level of heat was adjusted depending on the CO_2 flow rate. Since the heat applied to the metering valve was kept at a minimum possible level to maintain a consistent flow of CO_2, it is not anticipated to cause any carotenoid degradation.

The α- and β-carotene contents of the starting material determined with TSE were 524.2 and 611.4 μg/g (dry matter basis), respectively (Fig. 2.5a and 2.5b). Lutein was not recovered by the TSE method. The extraction yield with SC-CO_2 without canola oil addition for α-carotene was 137.8-330.4 μg/g and β-carotene was 171.7-386.6 μg/g feed material at the different temperature and pressure conditions tested, which was approximately half of the amount determined by TSE (Fig. 2.5a and 2.5b). The extraction yield with SC-CO_2 plus canola oil for α-carotene was 287.96-846.68 μg/g and β-carotene was 333.76-899.97 μg/g feed material, which was more than double that obtained without canola oil addition (Fig. 2.5a and 2.5b).

Pressure seemed to have a more substantial effect on the carotenoids yield at the higher level of oil addition (5%, w/w) to SC-CO_2 (Fig. 2.5d). At the conditions of higher temperature, pressure and canola oil concentration, the yields of carotenes with SFE were even higher than those obtained with TSE. In addition, a substantial amount of lutein was extracted by the SFE method at all the conditions studied, which was not possible with TSE. The lutein yield with SFE was 23.5-37.5 µg/g and 55.05-178.99 µg/g feed material from extraction without and with canola oil addition, respectively (Fig. 2.5c). These findings indicate that the use of canola oil as a co-solvent in SFE was very effective in increasing the carotenoids yield, which may be due to interactions between the canola oil and the carrot matrix, leading to the release of carotenoids from the matrix. As a co-solvent, canola oil may not only alter the polarity of the solvent mixture and therefore improve the solubility of the carotenoids in the SC-CO_2, but it may also have an effect on the carrot matrix. Canola oil may penetrate into the cell structure of dried carrots and make it swell. The fluid in the cells of the matrix may increase the pathlengths over which the carotenoids must travel to reach the external surface of the matrix. However, when canola oil swells the cell structure, as opposed to water in fresh carrots, it is easier for SC-CO_2 to penetrate and diffuse through the matrix due to the non-polar nature of both SC-CO_2 and canola oil. In addition, the swelling and loosening of the cell structure by canola oil may help release the carotenoids from the matrix, exposing them to a greater extent to the extraction solvent, thus enhancing extraction efficiency. By possibly competing for adsorption sites with the carotenoids, canola oil may enhance the release of caroenoids bound to the carrot matrix.

The estimated main effects of temperature, pressure and canola oil concentration on the yield of carotenoids were demonstrated in the Pareto chart presented in Figure 2.6, with the signs (positive or negative) indicating the effects. The vertical line represents the

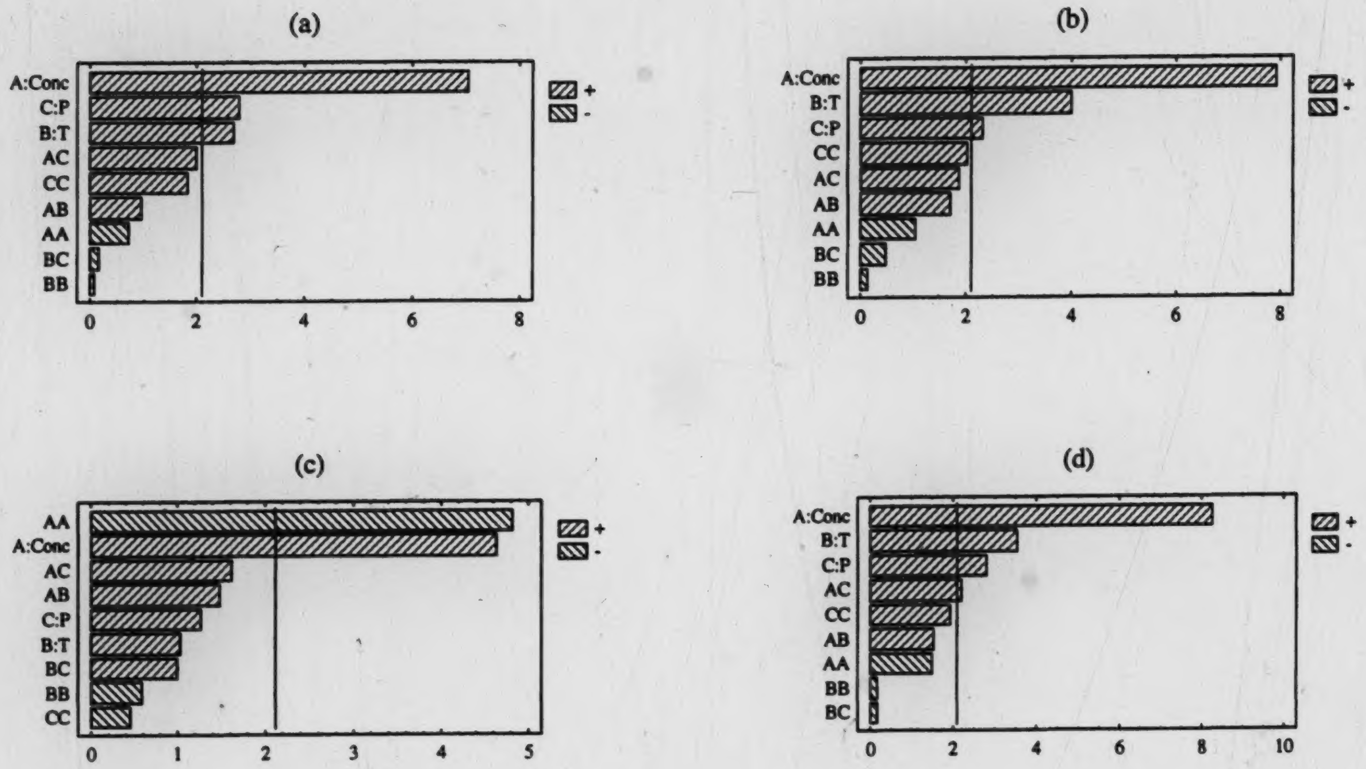

Figure 2.6. Standardized Pareto chart for carotenoids, (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids.
(A, B and C in the charts represent canola oil concentration, temperature and pressure, respectively, and AB, AC, BC, AA, BB and CC represent the interaction effects among temperature, pressure and canola oil concentration.)

critical limit to reach significant level ($p=0.05$). Those effects that have a larger absolute value than this limit are qualified as significant. For each of the carotenoids, only the main effects were significant. The plots of the main effects were shown in Figure 2.7 and the regression coefficients and the R-squared values were listed in Table 2.5. For α- and β-carotenes, canola oil concentration and temperature had significant ($p<0.05$) linear effect on their yields, while pressure had a significant ($p<0.05$) quadratic effect at low pressure level and a linear effect at high pressure level. The effect of pressure on β-carotene was less significant than that on α-carotene. When the SC-CO_2 extraction of carotenes from carrots with ethanol as co-solvent was studied, Vega et al. (1996) also reported that temperature and ethanol concentration had significant effects on the carotene yield and pressure had little or no effect. However, in a similar study, Barth et al. (1995) reported that temperature, pressure and ethanol concentration all had significant effects on the carotene yield and there were significant interactions among them as well. Yield of lutein was significantly ($p<0.05$) affected by canola oil concentration alone. Since β-carotene was the most predominant carotenoid among all the carotenoids extracted, the overall pattern for the total carotenoids followed those of β-carotene.

The two most significant effects of canola oil concentration and temperature on the yield of carotenoids were demonstrated in more detail in the three dimensional response surface plots presented in Figure 2.8. Although the behavior of the response plots was somewhat different at various pressures, the middle pressure of 41.3 MPa was adopted for the presentation in Figure 2.8. The pattern and the slope of the lines indicate

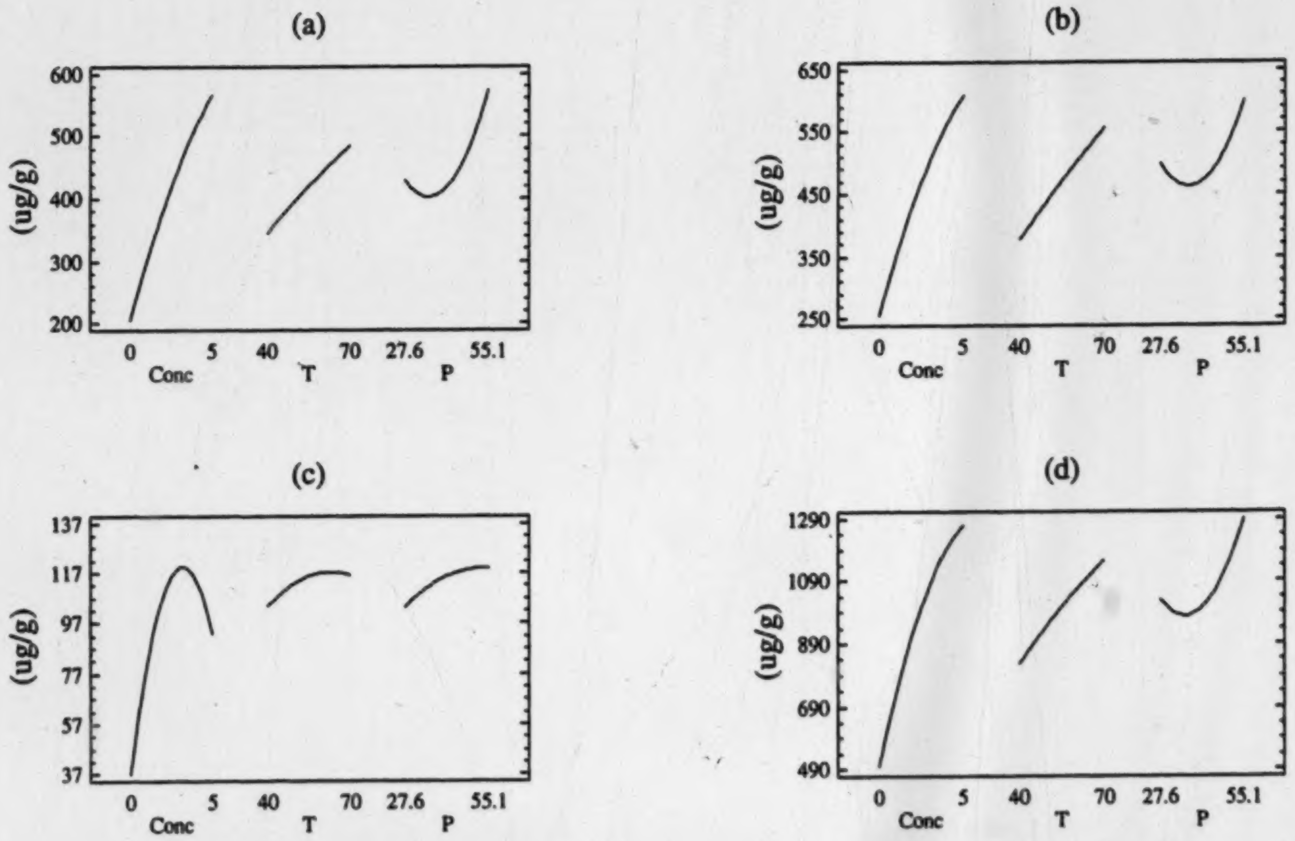

Figure 2.7. Main effects of temperature, pressure and canola oil concentration on the carotenoids yields, (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids.

Table 2.5. Regression coefficients and R-square for the fitted regression equations

	α-Carotene	β-Carotene	Lutein	Total carotenoids
Constant	667.2	601.417	28.6234	1297.18
T	5.5431	7.5261	1.0810	14.1497
P	0.2356	0.2106	0.0019	0.4451
Conc.	22.0326	26.7839	21.1030	27.7143
T*T	0.0179	0.0201	0.0261	0.0641
T*P	1.6653×10^{-3}	$4.1111*10^{-3}$	$2.4439*10^{-3}$	$3.3325*10^{-3}$
T*Conc.	0.8114	1.2279	0.2921	2.3315
P*P	$2.0528*10^{-5}$	$1.9458*10^{-5}$	$1.13847*10^{-6}$	$3.8849*10^{-5}$
P*Conc.	0.0125	0.0101	0.0024	0.0249
Conc.*Conc.	5.1124	6.1956	8.0592	19.3672
R^2	0.8122	0.8479	0.7576	0.8569

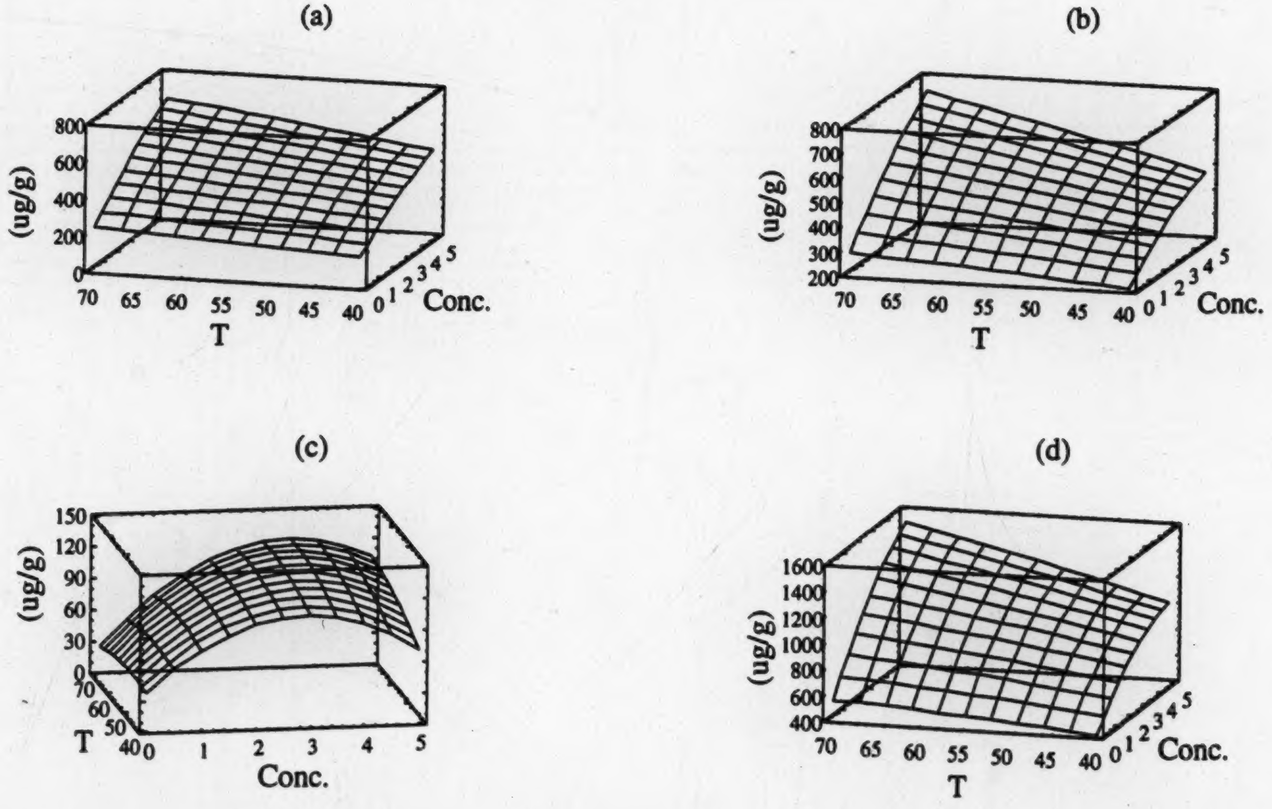

Figure 2.8. Estimated response surface plots for yield of carotenoids as a function of extraction temperature and canola oil concentration at 41.3 MPa, (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids.

that temperature did not have as significant an effect as canola oil concentration on the carotenoid yield. The yield increased with temperature at all concentration levels but this increase became more significant at higher oil concentrations. Barth et al. (1995) also showed that temperature had a positive effect on the carotene yield and the positive effect increased with ethanol concentration. But this result was only obtained at the low pressure (30.4 MPa) level. At mid pressure (40.5 MPa), the extraction yield of carotenes first increased then decreased with temperature at the low ethanol level, while at high ethanol concentration, the temperature effects on α- and β-carotene were different. Temperature showed no significant effect at high pressure (50.6 MPa) at either ethanol concentration (Barth et al., 1995). However, Vega et al. (1996) reported that the positive effect of temperature on the carotene yield diminished with increasing ethanol level. The concentration of canola oil had a positive quadratic effect at low temperatures and a positive but more linear effect at higher temperatures. Vega et al. (1996) also found a similar positive ethanol effect on carotene yield, which was quadratic at low and middle temperature levels and linear at the high temperature level. However, Barth et al. (1995) reported that ethanol had a positive effect only at the low pressure level. At mid pressure, the positive effect of ethanol could only be seen at high (50 °C) and low (30 °C) temperatures, while at mid temperature, ethanol showed a negative effect on the carotene yield. At high pressure (50.6 MPa), the effect of ethanol on the carotene extractability was diminished (Barth et al., 1995). Canola oil concentration was the only significant main effect on lutein yield and it showed a positive linear effect at low concentration and a negative quadratic effect at high concentrations. The optimum conditions for the maximum yield of each and total carotenoids within the experimental region studied were listed in Table 2.6.

2.3.3. Effect of particle size

The highest temperature (70 °C) and pressure (55.1 MPa) were proven to be the best temperature and pressure condition for the SC-CO_2 extraction of each individual and total carotenoids based on the results of Section 2.3.2; therefore, these conditions were adopted in the study of the effect of particle size on the extraction yield of carotenoids. For α- and β-carotene, 5% was the optimum canola oil addition level, while for lutein, 3.7% was the optimum condition; therefore, 5% canola oil addition was used as α- and β-carotene were the predominant carotenoids in the extract.

As shown in Figure 2.9, the total amount of oil collected was not affected significantly ($p>0.05$) with particle size. However, the carotenes extracted were significantly affected by particle size (Fig. 2.10a and 2.10b), with p values of 0.0086 and 0.0013 for α- and β-carotene, respectively. As expected, the smaller the particle size, the higher the amount of carotenoids extracted. A similar conclusion was drawn by Goto et al. (1987) when they worked with carotenoids extraction from carrots. This phenomenon is supported by the theory of solute diffusion in particles; the longer the distance a solute has to travel from inside of the particle to the surface of the particle, the slower the extraction. If a long enough time were allowed for the extraction, all of the carotenoids in the carrot matrix can be extracted eventually. Another important aspect is that there is a substantially larger contact surface per unit volume of extractor between the extraction solvent and carotenoids when a smaller particle size is used, which is especially

Table 2.6. The optimum extraction conditions for carotenoid extraction

Factor	α-Carotene	β-Carotene	Lutein	Total carotenoids
T (°C)	70	70	70	70
P (MPa)	55.1	55.1	55.1	55.1
Canola oil conc. (%)	5	5	3.76	5
Optimum value (μg)	872.55	905.03	139.1	1904.31

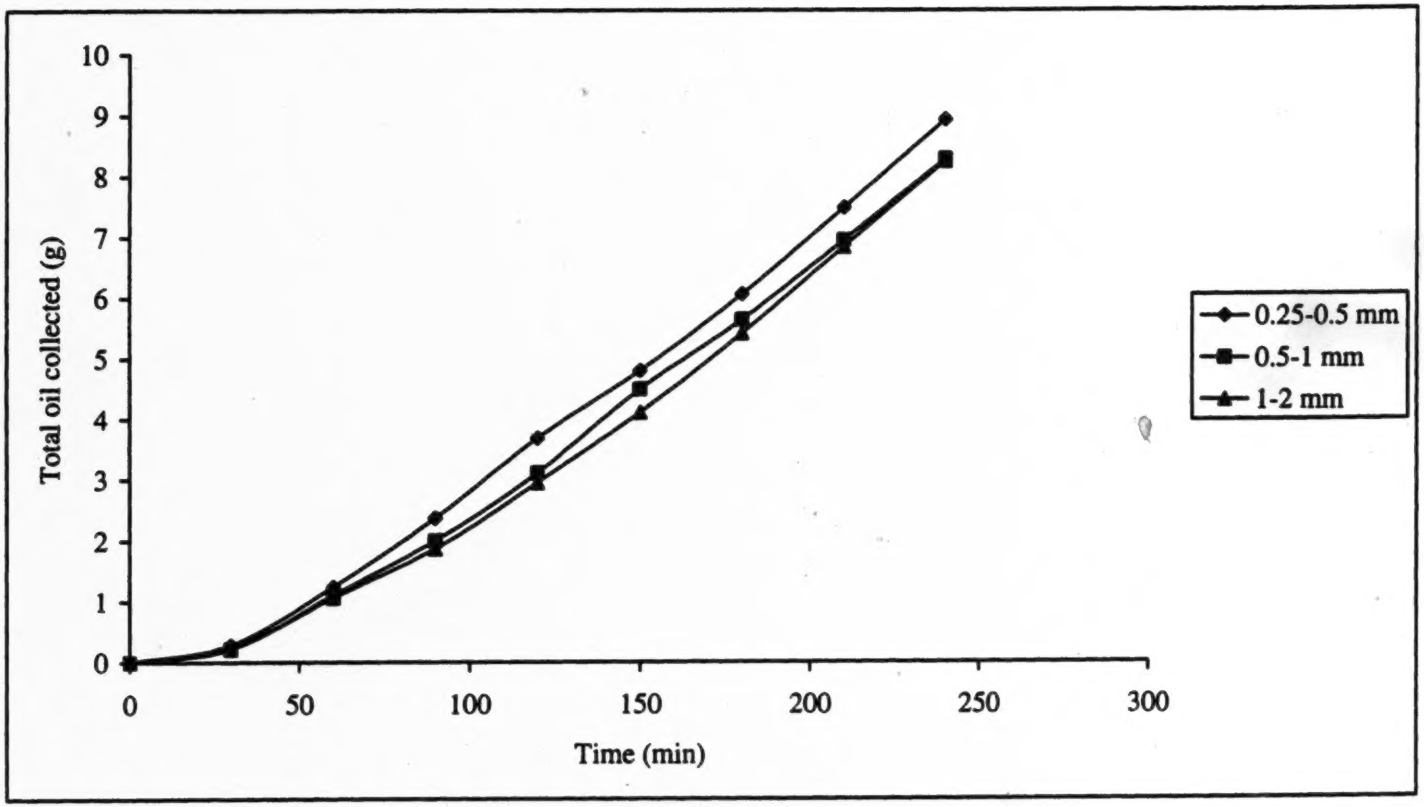

Figure 2.9. Total oil collected at different particle sizes
(dry feed sample extracted at 70 °C, 55.1 MPa, canola oil addition of 5% level and CO_2 flow rate of 1 L/min)

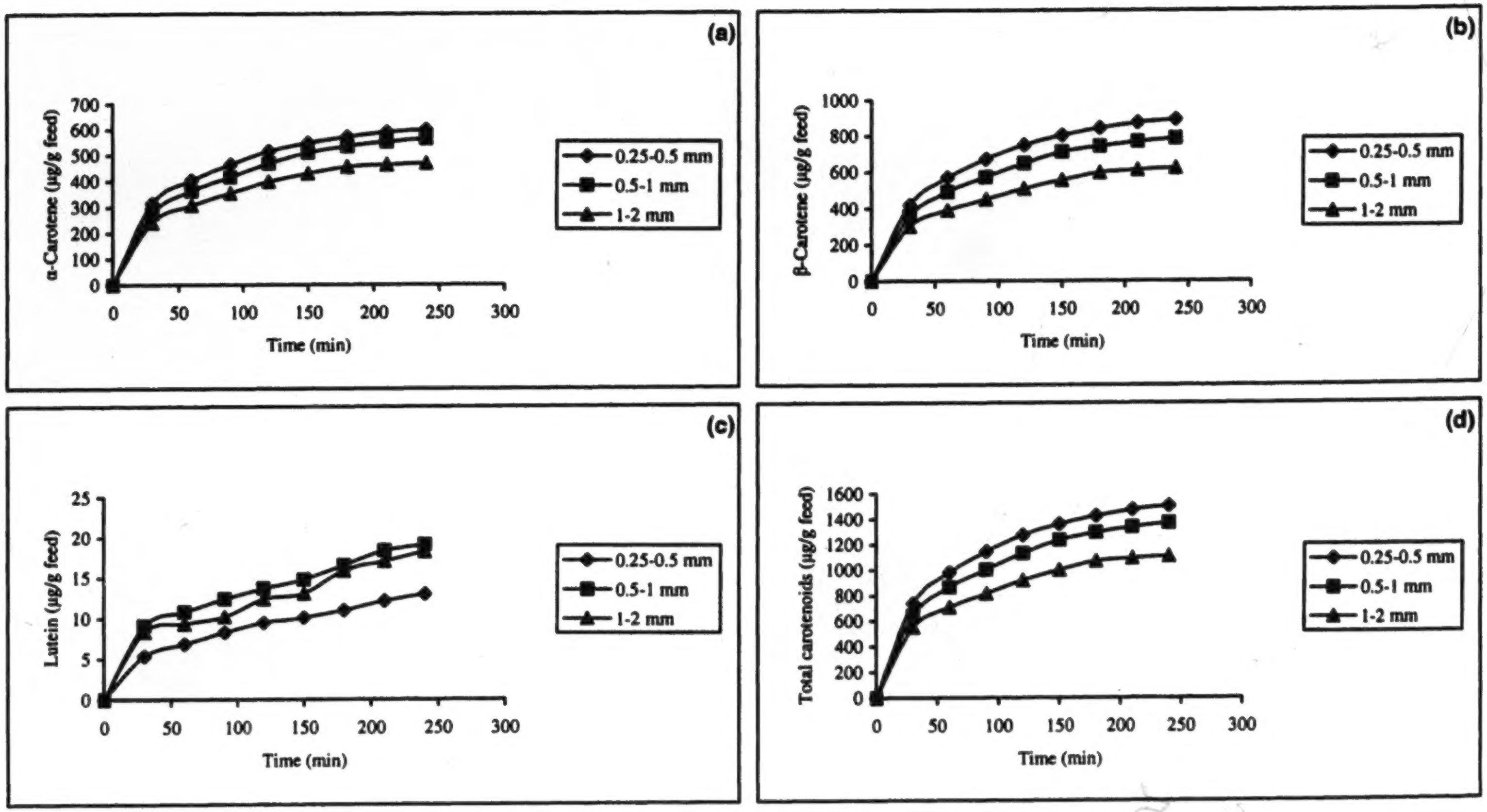

Figure 2.10. Carotenoids extracted (μg/g feed, dry matter basis) at different particle size (at 50 °C, 55.1 MPa and 5% canola oil concentration), (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids

important during the initial stages of extraction. From the fractions collected, it can be seen that most of the carotenoids was extracted during the first 30 min (Fig. 2.10). In the mean time, the total amount of sample recovered in the first fraction was less than that of the other 7 fractions. Even though the pumping of canola oil into the extraction cell was initiated from the moment the extraction was started, it required some time for the canola oil to saturate the carrot particles at a flow rate of 0.131 mL/min. Part of the added canola oil might be absorbed by the feed material while part of it was extracted with SC-CO_2 and the extraction efficiency for carotenoids was determined by the amount of oil in the cell as well as the solubility of canola oil in SC-CO_2. As extraction time progressed, there was more and more oil accumulated in the cell and the amount of oil extracted was completely determined by the solubility of oil in SC-CO_2. The effect of particle size on the lutein yield was not statistically significant ($p=0.086$). This might be explained by the fact that lutein has one hydroxyl group at each end of the β-ionone ring and, therefore, may bind to the cell structure components such as cellulose and pectins. Thus, lutein may not be as easily extracted with SC-CO_2 regardless of the particle size.

2.3.4. Effect of moisture content of feed material

Water represents approximately 80-90% of the total weight of plant material and interferes with the effectiveness of the SC-CO_2 extraction (Hopper and King, 1991). Therefore, drying is a necessary step prior to extraction. As carotenoids are sensitive to heat and oxygen, freeze-drying seems to be the choice of drying carrots prior to the SC-CO_2 extraction of carotenoids. However, freeze-drying is another expensive processing step in addition to SFE. To minimize the additional drying cost prior to SFE, finding the optimal level of drying of the feed material seems to be essential.

As the smallest particle size (0.25-0.5 mm) was found to be the best particle size in Section 2.3.3, this particle size was fixed in the study of the effect of moisture content of feed material on the carotenoids yield with SC-CO_2 extraction. The optimum temperature (70 °C), pressure (55.1 MPa) and canola oil concentration (5%, w/w) level were applied.

The moisture content of feed material had no significant ($p>0.05$) effect on the amount of total oil collected (Fig. 2.11), even though the total amount was slightly higher for the dry feed material. However, the amount of water collected in the extract was significantly affected ($p<0.0001$) by the moisture content of the feed material (Fig. 2.12). The higher the amount of water in the feed material, the higher was the amount of water co-extracted with oil and carotenoids. The slope of the extraction curve of the sample with 84.64% moisture content indicated that solubility of water in SC-CO_2 at 70 °C and 55.1 MPa was 2 mg/g. A similar water solubility in CO_2, 1.7 mg/g, was reported by King et al. (1992) at 40 °C and 10-20 MPa. The initial linear portion of the extraction curve of the carrot sample with a moisture content of 48.73% was parallel to that of the sample with 84.64% moisture content, indicating that the solubility limit for water in SC-CO_2 was reached. As the extraction process proceeded, less water was left in the sample and the solubility limit could not be reached, and therefore, the extraction curve levelled off.

The moisture content of the starting material significantly affected the carotenoids yield ($p<0.0001$ for all the individual carotenoids). The α- and β-carotene yield decreased

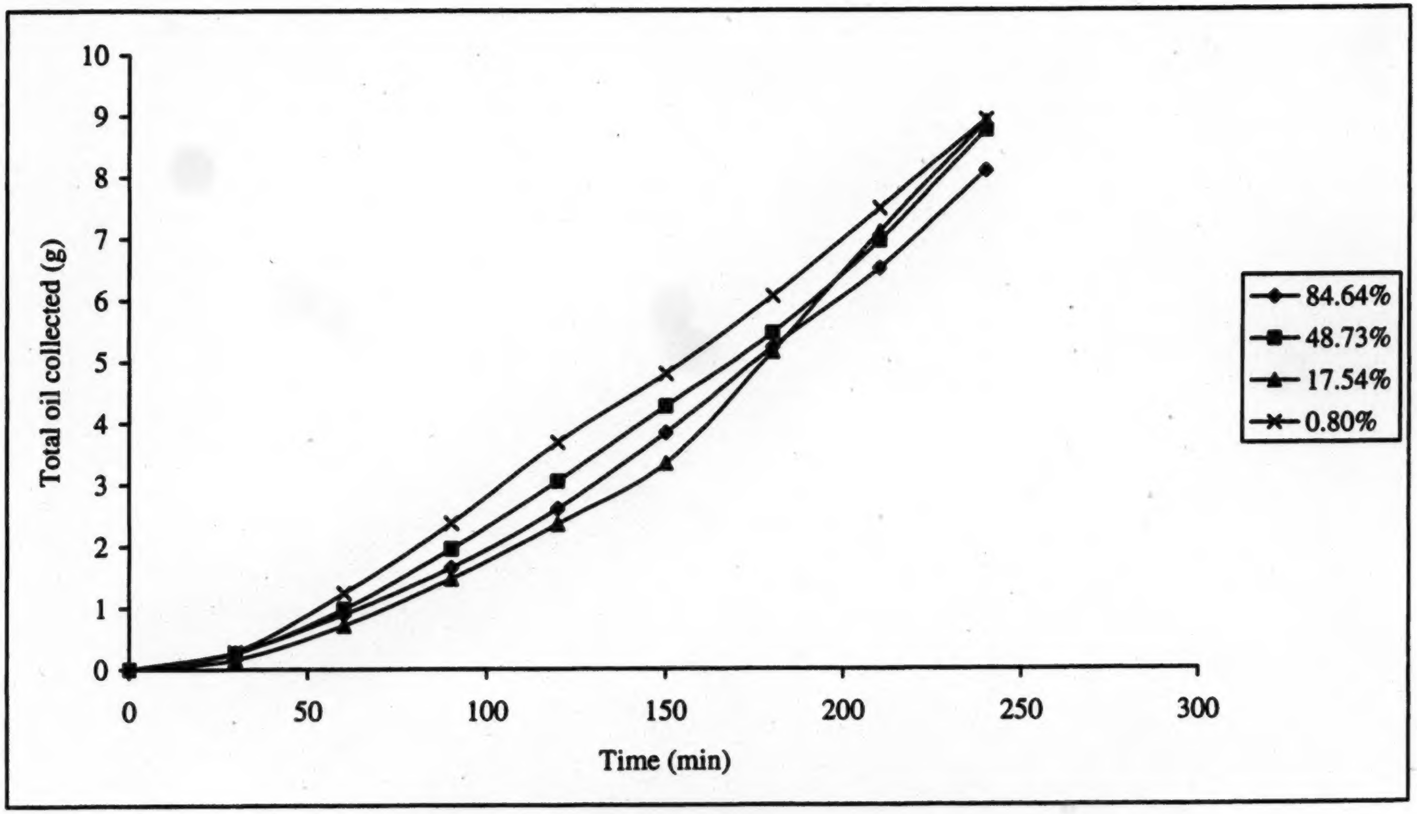

Figure 2.11. Total oil collected at different moisture levels
(extractions carried out at 70 °C, 55.1 MPa, canola oil addition of 5% level and CO_2 flow rate of 1 L/min)

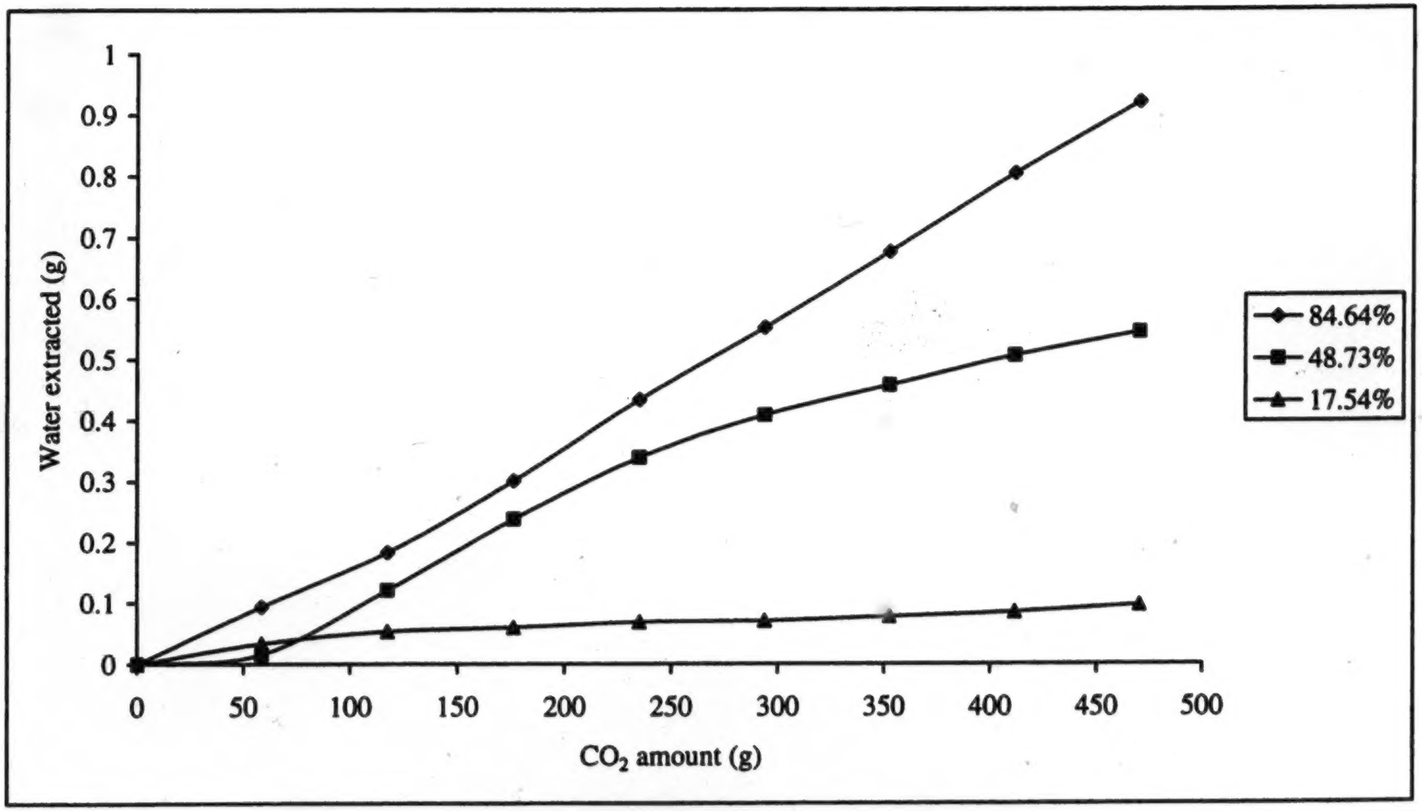

Figure 2.12. Water extracted at different moisture levels of feed material (extractions carried out at 70 °C, 55.1 MPa, canola oil addition of 5% level and CO_2 flow rate of 1 L/min)

with moisture, while lutein yield increased with moisture (Fig. 2.13). This can be explained by the fact that water can act as a co-solvent for the extraction of relatively polar compounds, whereas the presence of water is not favorable for the relatively non-polar carotenes. In addition, the presence of a large amount of water in the carrot matrix may decrease the extraction efficiency by increasing the pathlengths over which the carotenoids must travel to reach the bulk solvent. As well, the increased pathlengths were filled with a relatively polar component (water), which may increase the diffusion resistance of carotenoids, further decreasing the extraction efficiency. On the other hand, water may have the positive effect of swelling the matrix, which in this case may be less important than the negative effects discussed above. The fact that water reduced the extraction efficiency of β-carotene was also observed by Weathers et al. (1999). When a comparison of the SC-CO_2 extraction of 26 mg of dry and wet (spiked with 100 μL water) paprika samples were made, they found that the presence of water caused the extraction yield of β-carotene dropped to approximately half of that obtained from the dry sample. However, Goto et al. (1987) reported a complete extraction of carotenoids from raw carrot while the extraction of freeze-dried carrot was incomplete (70%). When added as a co-solvent, water neither significantly increased nor decreased the solubility of crystalline β-carotene in SC-CO_2 (Marsili and Callahan, 1993). The enhancing effect of a certain amount of water on the extraction yield of a relatively polar component in SC-CO_2 was also observed by Charest et al. (2001) and Leeke et al. (2002), when astaxanthin and essential oil were extracted, respectively. Leeke et al. (2002) explained that the

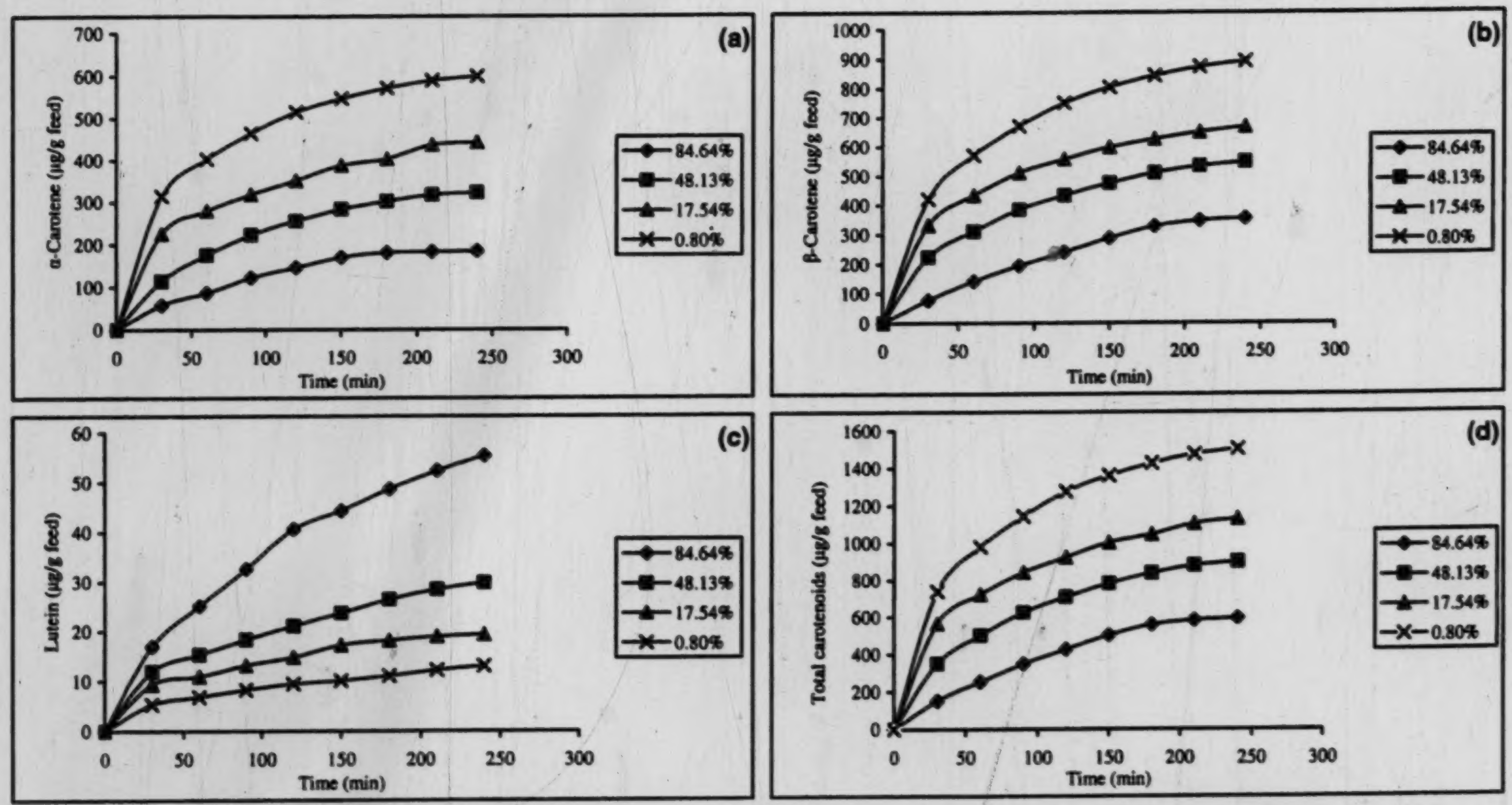

Figure 2.13. Caratenoids extracted (μg/g feed, dry matter basis) at different moisture levels of feed material (with particle size 0.25-0.5 mm at 70 °C, 55.1 MPa and 5% of canola oil addition), (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids

interaction between the functional groups of the oxygenated essential oil compounds and the water modifier was one of the factors that were responsible for the increased yield.

2.3.5. Effect of flow rate

Dry carrot particles were used in this section, since dried carrot was proven to give the highest carotenoids yield in Section 2.3.4. Other extraction conditions, including temperature of 70 °C, pressure of 55.1 MPa, canola oil addition at 5% (w/w) level and particle size of 0.25-0.5 mm were maintained. According to the previous studies conducted in our lab, the CO_2 flow rate at 1 L/min is low enough to allow the solute to reach its equilibrium solubility in SC-CO_2. However, a lower flow rate of 0.5 L/min was also tested for carotenoids for confirmation.

The total amount of oil collected increased with flow rate as shown in Figure 2.14. Figure 2.14a presented the extract amount as a function of extraction time while Figure 2.14b was as a function of the amount of CO_2 contacting the sample. At higher CO_2 flow rates, a larger amount of CO_2 is contacting the sample at any given time and thus a larger amount of oil is brought out of the system (Fig. 2.14a). However, when the amount of oil collected per g CO_2 is evaluated (Fig. 2.14b), it is seen that the loading of CO_2 is higher at lower flow rates approaching the solubility limit (28.2 mg/ g CO_2) at 0.5 L/min. The canola oil loading of CO_2 at flow rate of 1 L/min was 23.1 mg/g CO_2 and at flow rate of 2 L/min it was 17.9 g/g. The solubility value of canola oil in CO_2 obtained in this study, 28.2 mg/g, was between those reported by Lee et al. (1996) and Temelli (1992), which were 11 (at 55 °C and 36 MPa) and 43.3 mg/g (at 70 °C and 62 MPa), respectively, under somewhat similar extraction conditions.

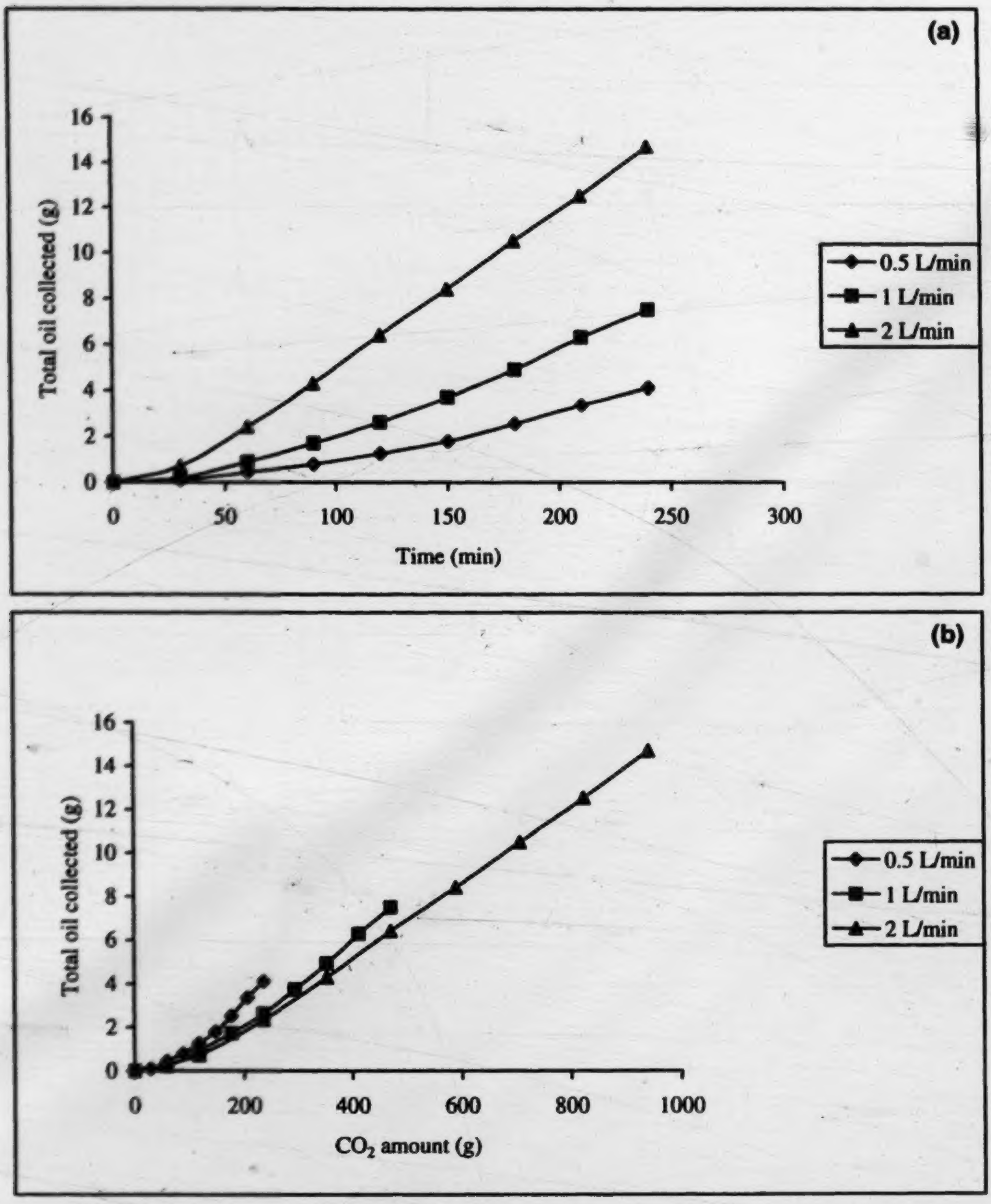

Figure 2.14. Total oil collected at different flow rates (at 70 °C, 55.1 MPa and 5% canola oil addition with 0.25-0.5 mm dry carrot particles), (a) total oil as a function of time, (b) total oil as a function of CO_2 amount

A high carotenoids yield was obtained at the high flow rate at the end of the 4 h extraction period (Fig. 2.15). As the flow rate was increased, the amount of carotenoids extracted also increased, since more carotenoids were solubilized with more fresh solvent. However, with a further increase in flow rate, the contact time between the carotenoids and the solvent becomes shorter and the solvent may leave the system without dissolving all the solute it can actually solubilize. In other words, the carotenoids may not reach their solubility limit before the solvent leaves the system. From Figure 2.15, it can be seen that at a flow rate of 0.5 and 1 L/min, β-carotene and lutein can reach their equilibrium solubility. The overlap of the extraction curves for total carotenoids at different solvent flow rates indicates that equilibrium solubility limit was achieved. Therefore, the slope of the linear portion of the extraction curve at the low flow rate was calculated as the solubility of the carotenoids in SC-CO_2 plus canola oil. The solubility of the total carotenoids was 5.96 μg/ g CO_2, while the solubility of α-carotene, β-carotene, and lutein was 3.03, 2.65, and 0.128 μg/g CO_2, respectively. The solubility of β-carotene in SC-CO_2 modified with canola oil from this study was the lower limit of the solubility range (2.69-9.11 μg/g CO_2) reported by Sovova et al. (2001), when grape seed oil was used.

2.4. CONCLUSIONS

Compared to the traditional solvent extraction method, not only the hydrocarbon compounds such as α-carotene and β-carotene but also the oxygenated carotenoids such as lutein were recovered by SC-CO_2 extraction. Canola oil, used as a co-solvent, can

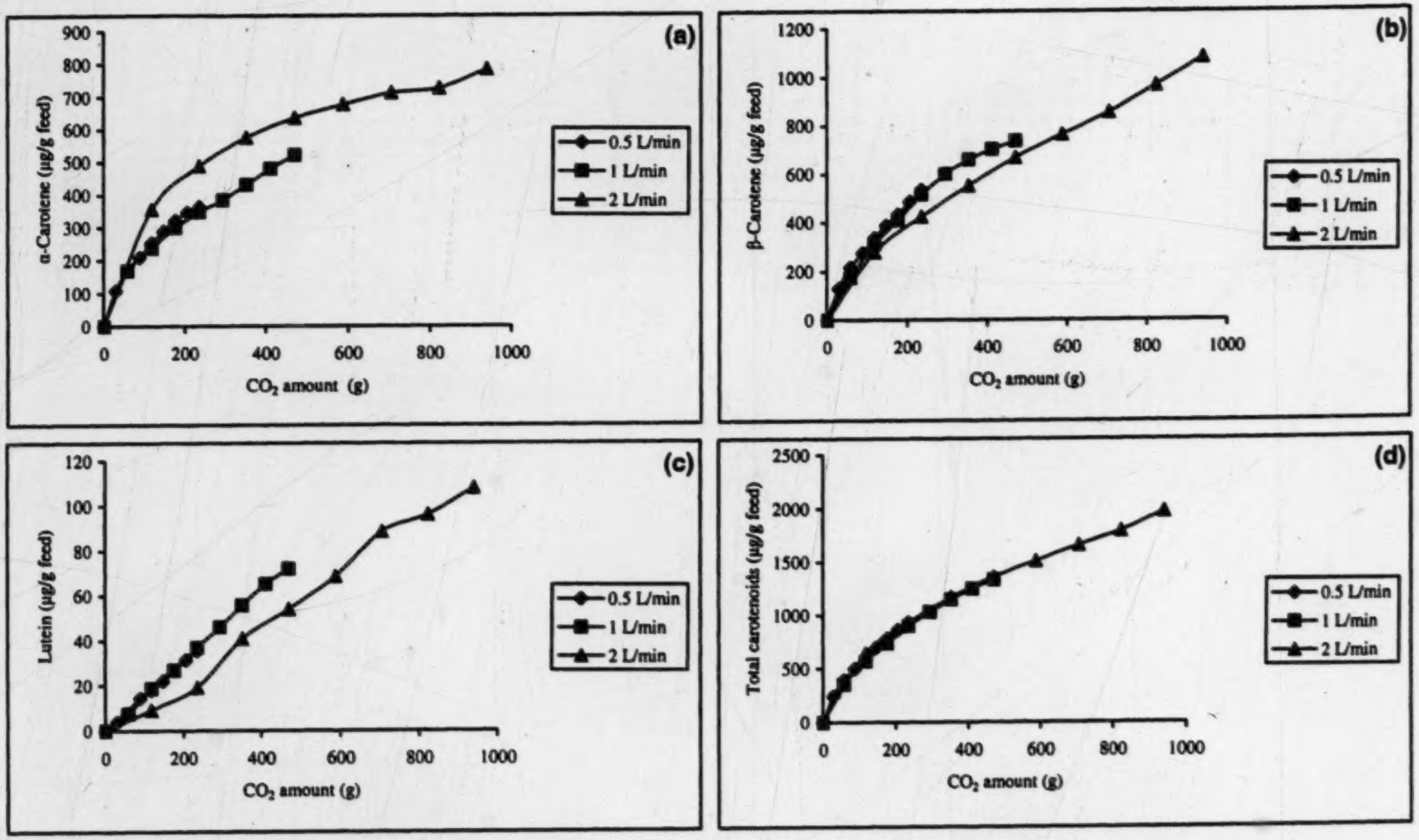

Figure 2.15. Amount of carotenoids extracted (µg/g feed, dry matter basis) at different CO_2 flow rates (at 70 °C, 55.1 MPa and 5% canola oil addition with 0.25-0.5 mm dry carrot particles), (a) α-carotene, (b) β-carotene, (c) lutein and (d) total carotenoids

greatly increase the extraction yield of carotenoids (for α- and β-carotene, by a factor of 2 and for lutein, by a factor of 4). In the region of experimental parameters studied, highest carotenoids yield was obtained from 0.25-0.5 mm dried carrot particles at the extraction conditions of 70 °C, 55.1 MPa, 5% canola oil addition (w/w), and 2 L/min (measured at ambient conditions) CO_2 flow rate. The extraction yield of total carotenoids with SC-CO_2 extraction under these conditions was substantially higher than that obtained with the TSE method. In summary, SFE is an efficient extraction technique for the recovery of "natural" carotenoids.

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3. CHARACTERIZATION OF CAROTENOID EXTRACT AND FIBRE RESIDUE FROM SC-CO_2 EXTRACTION OF CARROT[1]

3.1. INTRODUCTION

Health benefits of dietary fibre (DF) have been well documented. Today, many chronic disorders such as constipation, diverticulosis, cardiovascular diseases, diabetes and obesity are closely linked to the low fibre diet, especially in the developed countries. In the U.S. and Canada, the average dietary fibre intake is only 14-15 g/day (Best, 2001) and 14.6 g/day (Jenkins et al., 1985), respectively, which is well below the recommendation of the World Health Organization at 25-40 g/day (Jalili, 2000). Due to the health problems associated with low fibre diets, dietary fibre plays an important role in the modern nutraceutical and functional foods field (Pszczola, 1998; Bollinger, 1999; Jalili, 2000). Recently, the U.S. Food and Drug Administration has approved a health claim regarding "the relationship between the fruits, vegetables and grain products that contain fibre, particularly soluble fibre and coronary heart disease" (Heller et al., 1999). The dietary fibre ingestion can be increased either by changes in the eating habits or by supplementation of commercial fibre-rich products. The milling by-products of grains such as wheat bran have been traditionally used as fibre supplement. However, wheat bran was reported to produce mineral deficiencies (Reinhold et al., 1976). Even though there seems to be a controversy on determining which component is responsible for reducing mineral availability, the quality of the dietary fibre becomes a nutritional factor, particularly concerning the phytate content. Dietary fibre from fruit and vegetable sources contains relatively low amounts of phytate, high ratio of water-soluble to water-insoluble fibre, and most importantly, some specific bioactive constituents such as polyphenols, flavonoids and carotenoids. Therefore, there is an increasing interest to find new sources of dietary fibre.

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[1] A version of this chapter is to be submitted to the *J. Agric. Food Chem.* for consideration for publication.

The interest in studying DF is not only in the knowledge of the amount of DF in foods but also in understanding the physiological effects that it may have on humans. The physiological effects of a particular fibre in the gastrointestinal tract are determined by its chemical and physical characteristics. Beyond the basic nutritional considerations, the functional properties of dietary fibre such as water binding capacity (WBC) are also an important concern for food scientists, as these characteristics are intimately related to how dietary fibre functions as an ingredient in a food product formulation, product yield and shelf stability of formulated products. Over the past decade, fruit DF concentrates (lemon, apple and citrus) have been introduced into the market dominated by cereal-based DF ingredients. Many different kinds of fruits such as peach, pineapple, grape and orange (Saura-Calixto, 1998; Grigelmo-Miguel and Martin-Belloso, 1999; Grigelmo-Miguel et al., 1999; Prakongpan et al., 2002) and vegetables such as carrot, onion, cauliflower, potato, beans and mushroom (Toma et al., 1979; Konno et al., 1986; Phillips and Palmer, 1991; Svanberg et al., 1995; Li and Zhao, 1997; Kim, 1998; Femenia et al., 1999; Cheung and Lee, 2000; Jaime et al., 2002) have been studied either for the characterization of fibre or for their applications. However, no detailed information is available for the physical properties of carrot fibre, especially its antioxidant capacity and color properties.

An antioxidant is a substance that inhibits oxidation or inhibits reactions promoted by oxygen or peroxides. Carotenoids are the main antioxidant in carrot. Carotenoids have been extracted from many sources such as carrot, tomato, paprika, sweet potato, algae and pressed palm oil (Spanos et al., 1993; Barth et al., 1995; Mendes et al., 1995; Vega et al., 1996; Jaren-Galan et al., 1999; Baysal et al., 2000; Franca and Meireles, 2000) by supercritical CO_2 (SC-CO_2), a unique fluid, with which a solvent-free and high purity "natural" extract can be produced, as compared to the traditional organic solvents. However, there are no studies reporting about the antioxidant activity of carotenoids extracted from carrot using SC-CO_2.

Among the appearance properties of foods, color is a key attraction to consumers, as it conveys a concept of readiness for consumption. To be a qualified food ingredient, fibre should possess a color as light as possible. The SC-CO_2 extraction parameters that have been studied in Chapter 2, including temperature (T), pressure (P), canola oil co-solvent concentration, particle size and moisture content of the feed material and CO_2 flow rate, had an effect on the yield of carotenoids, which are responsible for the color of carrots. Therefore, the extent of carotenoid extraction should also have an impact on the color of the fibre residue.

Therefore, the objectives of this study were:

1. to determine the DF content and to evaluate some of the most important physico-chemical properties of carrot fibre residue, a by-product of SC-CO_2 extraction of carotenoids,

2. to investigate the antioxidant activity of the carotenoid-containing extract products, and
3. to evaluate the effect of T, P, and canola oil concentration, flow rate, particle size and moisture content of the feed material on the color parameters of extraction fibre residue.

3.2. MATERIALS AND METHODS

3.2.1. Materials

The carrots (*Dutch narbonne*), which were used for the investigation of the antioxidant activity of the carotenoids-enriched oil extract and the physico-chemical properties of the carrot fibre residue, were supplied in April, 2003 by the Kuhlmann's Market Gardens & Greenhouses, Edmonton, AB. The carrots were seeded in the middle of May and harvested in the middle of October, 2002. After harvest, the carrots were stored at 0 °C without any relative humidity control of the storage atmosphere.

The extraction residue products obtained from the extractions carried out in Chapter 2 (Section 2.2.5) were used for the evaluation of the effects of the extraction parameters on the color parameters of the carrot fibre residue.

Canola oil, ethanol, acetone, hexane were all from the same sources as described in Chapter 2 (Section 2.2.1). Ferrous chloride was obtained from Fisher Scientific (Fair Lawn, NJ). Hydrochloric acid was analytical grade and from BDH Inc. (Toronto, ON). Butylated hydroxytoluene (BHT), linoleic acid, and ammonium thiocyanate were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium phosphate was from J. T.

Baker Chemical Co. (Phillipsberg, NJ). Micro cleaning solution was bought from the International Products Corp. (Trenton, NJ). Celite, α-amylase (3000 ceralpha Units/mL), protease (350 Tyrosine Units/mL) and amyloglucosidase (200 p-NP β-maltoside Units/mL) were obtained from the Megazyme International Ireland Limited (Co. Wicklow, Ireland). Two-(N-morpholino)-ethanesulfonic acid (MES) and tris (hydroxymethyl) aminomethane (TRIS) were bought from Sigma Chemical Co. (St. Louis, MO).

3.2.2. Sample preparation and extraction

The carrots (10 kg) were washed and peeled after receiving. The peeled carrots were chopped into 5 mm cubes at room temperature with a food chopper (Model 84185, Hobart Corporation, Troy, OH). The carrot cubes were well mixed, spread on trays and freeze-dried (Model FFD-42-WS, The Virtis Co. Inc., Gardiner, NY) for 5 days. The carrot cubes were vacuum packed in moisture and O_2-barrier bags, then labeled and stored at -18 °C in dark.

The carrot fibre and oil extract were obtained using the same supercritical fluid extraction unit described in Chapter 2 (Section 2.2.5) except that the sample loading basket was replaced with a larger one (24.6 cm × 26 mm I.D.) to accommodate the larger sample size. The extraction was carried out with carrots of particle size in the range of 0.25 to 0.5 mm. The desired particle size was achieved by grinding the carrot cubes using a Quadro Comil grinder (Model 197S, Emerson Industrial Controls, Grand Island, NY) and sieving (The W. S. Tyler Company of Canada Ltd., St. Catharines, ON) prior to extraction. Ten extractions were carried out at 70 °C, 55.1 MPa, 5% canola oil addition as a co-solvent and 2 L/min CO_2 flow rate (measured at ambient conditions). Approximately 20 g carrot sample was loaded into the extraction cell for each extraction. Each extraction was continued for 4 h with canola oil addition, followed by 3 h extraction with CO_2 alone to recover the remaining canola oil introduced into the system. The ten oil extracts were combined and the crystallized carotenoids were separated from the carotenoid-saturated canola oil by centrifugation for further testing of their antioxidant activity. The fibre residues obtained from the ten extractions were well mixed for further characterization of its physical and chemical properties.

3.2.3. Chemical analysis

3.2.3.1. Proximate composition

Moisture, ash, protein, crude lipid and carbohydrate contents of the raw material were determined as described in Chapter 2 (Section 2.2.3).

3.2.3.2. Carotenoids

Carotenoids content of raw material was determined as described in Chapter 2 (Section 2.2.4).

3.2.3.3. Fibre

Fibre analysis was conducted on the SC-CO_2 extraction residue of carrot according to the AOAC Offical Method 991.43.2 (AOAC, 2000). Total dietary fibre (TDF) was determined as the sum of insoluble dietary fibre (IDF) and soluble dietary fibre (SDF).

3.2.3.3.1. Preparation of crucibles

Fritted disks (coarse, ASTM 40-60 µm pore size, pyrex 60 mL) were ashed overnight at 525 °C in muffle furnace (Model F-A1730, Thermolyne Corporation, Dubuque, IA). The crucibles were soaked for 1 h in 2% micro cleaning solution at room temperature, then rinsed with water, deionized water and 15 mL acetone in sequence and air-dried. Approximately 1.0 g celite was added to the dry crucibles and dried at 130 °C until constant weight was reached. The crucible with celite was cooled in a desiccator and weighed to nearest 0.1 mg.

3.2.3.3.2. Test sample preparation

Two blanks were run in parallel with the test sample to determine any contribution from the reagents to the fibre residue. Approximately 1.0 g carrot sample was weighed in duplicate into 400 mL tall-form beakers. Forty milliliter MES-TRIS buffer (pH 8.2) was added to each beaker and stirred on magnetic stirrer until the carrot sample was completely dispersed. Fifty microliter thermostable α-amylase solution was added to each beaker while stirring at low speed and the mixtures were incubated in 95-100 °C water bath for 35 min with continuous agitation. When the beakers were cooled to 60 °C, any sample ring formed inside the beaker was scraped and any lumps of gel formed at the bottom were dispersed with 10 mL milli-Q water. Then, 100 µL protease solution was added to each beaker and the mixtures were incubated for 30 min at 60 ± 1 °C with continuous agitation. Five milliliter of 0.561 N HCl solution was dispersed into the mixture while stirring, followed by the measurement of pH, which should be between

4.1-4.8. Finally, 200 μL amyloglucosidase solution was added while stirring and the mixtures were incubated for 30 min at 60 ± 1 °C with constant agitation.

3.2.3.3.3. Insoluble dietary fibre determination

The crucible containing celite was weighed to nearest 0.0001 g and the celite bed was wetted and redistributed using approximately 3 mL Milli-Q water. Suction was applied to the crucible to draw celite onto the fritted glass as an even mat. The enzyme digestate was filtered through the crucible into a filtration flask. The beaker was rinsed and the residue was washed twice with 10 mL 70 °C water. The filtrate and water washings were combined, transferred to a beaker and reserved for the determination of soluble dietary fibre. The residue was washed twice each with 10 mL of 95% ethanol and acetone under vacuum. The crucible, containing the residue was dried overnight in 103 °C oven, cooled in a desiccator and weighed to 0.0001 g. One of the duplicates from each test portion was used to determine the protein content as described in Chapter 2 (Section 2.2.3.3). The other duplicate sample was used for ash analysis as described in Chapter 2 (Section 2.2.3.2). The weight of the crucible and celite was subtracted to determine ash weight.

3.2.3.3.4. Soluble dietary fibre determination

The beaker with combined solution of filtrate and water was weighed and the solution volume was estimated. Four volumes of 95% ethanol preheated to 60 °C was added to the solution. Filtering flask used in IDF determination was rinsed with portions of 60 °C ethanol. The solution was left at room temperature for 1 h to precipitate. The crucible, containing celite was weighed to nearest 0.0001 g, wetted and redistributed using 15 mL 78% ethanol from wash bottle. Suction was applied to the crucible to draw celite onto the fritted glass as an even mat. The alcohol-treated enzyme digestate was filtered through the crucible. All the remaining particles were quantitatively transferred to the crucible using a wash bottle with 78% ethanol and a rubber spatula. The residue was washed twice, each with 15 mL portions of 78% ethanol, 95% ethanol, and acetone under vacuum. The crucible, containing the residue was dried overnight in 103 °C oven, cooled in a desiccator and weighed to nearest 0.1 mg. The residue weight was calculated by subtracting the weight of dry crucible with celite. The duplicates were used to determine protein and ash as described in insoluble dietary fibre preparation (Section 3.2.3.3.3).

3.2.3.3.5. Calculations

Blank (B, mg) determination:

$$B = \frac{BR_1 + BR_2}{2} - P_B - A_B \tag{3.1}$$

where BR_1 and BR_2 were residue weights (mg) for duplicate blank determinations, and P_B and A_B were the weights of protein and ash, respectively, determined on the first and second blank residues, respectively.

Dietary fibre content (DF, g/100g) determination:

$$DF = \frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} * 100 \tag{3.2}$$

where R_1 and R_2 were residue weights (mg) for duplicate test portions, P and A were weights (mg) of protein and ash, respectively, determined on the first and second residues, B is blank weight (mg) and M_1 and M_2 were weights (mg) for test portions.

3.2.4. Physical properties

3.2.4.1. Particle size and size distribution

Particle size distribution of the carrot fibre was determined using a set of standard sieves (16, 32, 35, 60, 80, and 120 mesh). Sixty grams of sample was placed on the largest of the descending size pre-weighed sieves, which were fitted with a pan at the bottom and a cover on top. The "nested" sieves were shaken mechanically for 10 min, then re-positioned and shaken for an additional 5 min. The weights of the sieve plus sample retained on each sieve were recorded. The particle size distribution was expressed as the percentage of weight of particles retained on each sieve of the original sample weight.

3.2.4.2. Density

Bulk and packed densities were determined as follows:

3.2.4.2.1. Bulk density

Three 100 mL graduated cylinders were pre-weighed. Carrot samples were added to the cylinders with slight shaking by hand. The weights of cylinder and sample were recorded when the sample volumes reached 100 mL. The bulk density was expressed as weight per unit volume.

3.2.4.2.2. Packed density

Three 10 mL calibrated syringes were pre-weighed and then filled with a certain amount of carrot fibre residue. The weights of syringe and fibre were recorded. Pressure was applied manually to the samples until additional pressure would not further reduce the sample volume and the volumes were recorded. The packed density was calculated as weight of sample per pressed volume.

3.2.4.3. Water binding capacity

The water binding capacity (WBC) was determined according to the AACC method 88-04 (AACC, 1995).

3.2.4.3.1. Determination of approximate WBC

Approximately 5.0 g test material (as-is) was weighed into a pre-weighed 50 mL centrifuge tube. Milli-Q water was added in small unmeasured increments and the sample was stirred with a glass rod after each addition until the mixture was completely wetted. The stirring rod was wiped on the side of the tube. The sample was centrifuged at 2,000 × g for 10 min (Model J2-21, Beckman Instruments, Inc., Palo Alto, CA). The water addition and centrifugation steps were repeated until there was apparent separation of supernatant. The supernatant was discarded and the tube with the sediment was weighed. The approximate WBC was calculated as follows:

$$\text{Approx. WBC} = ((\text{wt. of tube} + \text{sediment}) - (\text{wt. of tube} + 5.0))\ \text{mL}/5\ \text{g} \qquad (3.3)$$

3.2.4.3.2. Determination of WBC

The quantity of material calculated as follows was weighed into each of four centrifuge tubes:

$$\text{wt. of material} = 15/(\text{approx. WBC} + 1) \qquad (3.4)$$

where, 15 was the desired total weight of sample and water. Volumes of water, 1.5 and 0.5 mL more and 1.5 and 0.5 mL less than that calculated by (15 - wt. of material) were added to the four tubes. The content of each tube was stirred vigorously with a stirring rod for 2 min, and then centrifuged as before. The two adjoining tubes, one with and one without supernatant, represent the range in which the WBC value occurs. The WBC value (mL/g) is presented as the midpoint between these two volumes divided by sample weight.

3.2.4.4. Swelling

The swelling capacity was determined according to a modified method of Auffret et al. (1994). Approximately 2 g sample was weighed in three 25 mL graduated cylinders. The volumes of the sample were recorded. Then, 30 mL distilled water was added to each of the cylinders and stirred with a glass rod. The samples were left at room temperature for 24 h and then the volume of the carrot residue settled in the cylinders was recorded. The swelling volume was calculated as the volume increase per unit weight of sample.

To have an indication of the volume expansion of the sample that holds the maximum amount of water, the sample after swelling capacity test was pressed by the piston of a 10 mL syringe until no further reduction in volume was obtained, and the pressed volume was recorded. The volume increase per gram of sample after hydration and pressing was calculated.

3.2.4.5. Oil binding capacity

The oil binding capacity (OBC) was determined according to Ang (1991). Two grams of carrot residue fibre were weighed in duplicate in pre-weighed 50 mL centrifuge tubes. The samples were mixed with 30 mL canola oil using a glass rod. The samples were centrifuged at 2,000 × g for 15 min. After centrifugation, the supernatant solution was drained and the weight of tube and wet sample was recorded. The oil binding capacity was expressed as the weight of oil retained per unit weight of sample.

3.2.4.6. pH

The pH of the 10% (w/v) carrot fibre residue solution was determined potentiometrically with a pH meter (220 pH meter, Corning Science Products, Corning, NY) in triplicate.

3.2.4.7. Energy

The energy value of the carrot fibre was determined by combustion with the aid of a calorimetric pump (Autobomb, Gallenkamp, UK) in triplicate.

3.2.4.8. Color

The color parameters of extraction residues and feed material were measured. A Labscan XE spectrocolorimeter (Model LSXE/UNI, Hunterlab Associates Laboratory, Inc., Reston, VA) was used to measure 'L', 'a' and 'b' values of carrot extraction residues, of which 'L' indicates brightness/darkness, 'a' indicates redness/greenness and 'b' indicates yellowness/blueness. The LabScan XE sensor was calibrated with a white tile ('L' = 91.66, 'a' = -1.0 and 'b' = -0.2). Hue was expressed as 'a' / 'b'. Chroma was expressed as $\sqrt{a^2+b^2}$. The overall color change (ΔE) of carrot particles before and after extraction was expressed as

$$\Delta E=\sqrt{(L_1-L_0)^2+(a_1-a_0)^2+(b_1-b_0)^2} \qquad (3.5)$$

3.2.4.9. Antioxidant activity

Two grams of carrot residue fibre was extracted in triplicate by the traditional solvent extraction method as described in Chapter 2 (Section 2.2.4). After washing three times with 100 mL milli-Q water, the organic phase was dried using a rotavapor (Model RE 21, Buchi Laboratoriums-technik, AG, Flawil, Switzerland).

The antioxidant activity of the fibre residue extract was determined along with those of carotenoid crystals and oil sample by the ferric thiocyanate (FTC) method reported by Larrauri et al. (1996). A mixture of 0.5 mL of a weighed sample in absolute ethanol, 0.5 mL of 2.51% linoleic acid in 99.5% ethanol, 1 mL of 0.05 M sodium phosphate buffer (pH 7.0) and 0.5 mL of distilled water was placed in a small vial with a screw cap, then shaken and placed in an oven at 40 °C in dark. A control without any test sample extract was also used. To 0.1 mL of this solution was added 9.7 mL 75% ethanol and 0.1 mL 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the sample was measured using a spectrophotometer (Model Spectronic 3000 array, Milton Roy Company, Rochester, NY) against a reagent blank at 500 nm, every 24 h (t) until one day after the absorbance of the control reached maximum. The oxidation index (OI) and antioxidatant activity (AA) were calculated as:

$$\mathrm{OI} = 100 \times (\mathrm{Absorbance}_t \,/\, \mathrm{Absorbance}_{t=0}) \tag{3.6}$$

$$\mathrm{AA} = 100 - 100 \times (\mathrm{Product\ oxidation\ index}_t \,/\, \mathrm{Control\ oxidation\ index}_t) \tag{3.7}$$

3.2.4.10. Viscosity

The viscosity of the 5% (w/w) carrot fibre in milli-Q water solution processed at three different temperatures was tested with the universal dynamic spectrometer (Model UDS 200, Paar Physica USA, Glen Allen, VA). After mixing on vortex (Model G-560, Scientific Industries, Inc., Bohemia, NY) at high speed for 30 s, one set of solutions was left at room temperature for 10 min and the other two sets were cooked at 50 and 80 °C on hot plate (Model 310T, Fisher Scientific, Fair Lawn, NJ), for 10 min. Then the samples were centrifuged (Model J2-21, Beckman Instruments, Inc., Palo Alto, CA) for 15 min at 10,000 × g. The supernatant was transferred to the Paar Physica sample cup and the viscosity was measured at 20 °C as a function of shear rate.

3.2.4.11. Gel formation

The gel forming property was tested with 5% (w/w) carrot fibre in milli-Q water. The samples were mixed at high speed for 30 sec and then cooked at 50 and 80 °C, respectively, for 30 min. The cooked samples were kept at 4 °C overnight to test the gel forming property.

3.2.5. Statistical analysis

Of the different physical properties of carrot fibre residue investigated, particle size and size distribution, water binding capacity, oil binding capacity, viscosity and gel formation were all determined in duplicate, while the density, swelling capacity, pH, energy, color and antioxidant activity were all determined in triplicate and the mean values were reported. The antioxidant activity of carotenoid crystals and carotenoid-saturated canola oil were determined in duplicate. The effects of temperature, pressure and canola oil concentration on the color of residue were analyzed by STATGRAPHICS Plus Version 5 (Manugistics Inc., 2000). The effects of particle size, flow rate and moisture content of the feed material on the color parameters were analyzed by the General Linear Model procedure of SAS Statistical Software, Version 8 (SAS Institute Inc., 1999).

3.3. RESULTS AND DISCUSSION

3.3.1. Chemical analysis

3.3.1.1. Proximate composition and carotenoid content

The proximate composition and dietary fibre and carotenoid content of the raw carrots used in this study were listed in Table 3.1. Compared with the composition of carrots used in Chapter 2 (Table 2.4), this batch of carrots had a higher protein, lipid and ash contents and a lower moisture and carbohydrate contents. The total carotene contents (no lutein was recovered by TSE) of these two batches were similar, but the ratio of α- to β-carotene was much higher in the previous batch (Table 2.4). This might be due to the differences in variety and growing conditions of the raw carrots, since one batch was produced in the U.S. and one in Canada.

3.3.1.2. Dietary fibre content

The total dietary fibre content of the carrot residue fibre obtained after SC-CO_2 extraction of carotenoids was 22.4%, with 17.64% of IDF and 4.76% of SDF. The removal of the peel of carrot would contribute to a lower fibre content. The total dietary fiber content of fresh and frozen carrots were reported to range from 1.98% to 2.41% based on the wet weight, of which, the IDF cellulose and lignin accounted for 0.77-0.97% and 025-0.39%, respectively, and the SDF pectins accounted for 0.72-1.06% (Ross et al., 1985). The DF content of the hot air dried carrot juice residue reported by Kim (1998) was higher, 44.57% total fibre, 24.81% and 19.76% for IDF and SDF, respectively. In Kim's study, the carrot juice residue was not pretreated to remove sugar, protein and lipids. Therefore, the dietary fibre content could have been even higher if those

Table 3.1. Composition of fresh carrots

Proximate composition (g/100 g)[1]	Moisture	89.17±0.07
	Ash	0.55±0.00
	Protein	0.72±0.01
	Lipid	0.058±0.0007
	Carbohydrate	10.27
Total dietary fiber (g/100 g)[2]	Insoluble dietary fiber	1.91
	Soluble dietary fiber	0.52
Carotenoids (μg/100 g)[2]	α-Carotene	3466±383
	β-Carotene	7879±612

[1] Means ± standard deviation based on triplicate determinations

[2] Means ± standard deviation based on duplicate determinations

components were removed. When the carrot fibre of this study was compared to fibres from other sources (Table 3.2), the fibre content and the insoluble to soluble fibre ratio were found to be similar to those of oat bran.

3.3.2. Physical properties of extraction residue

3.3.2.1. Particle size and size distribution

The particle size distribution of the carrot fibre was shown in Table 3.3. The particle size of the carrot fibre was mainly between 250 and 425 μm, with a small portion of particles in between 425-500 and 180-250 μm. This centered particle size is caused by the grinding and sieving carried out prior to the extraction process. The effect of temperature on fibre particle size was studied by Parrott and Thrall (1978). They found that when the water temperature was increased from 20 °C to 100 °C, some fibres had enlarged particles, some fibres had swollen individual cells and some fibres had a collapsed structure.

3.3.2.2. Density

The bulk density of the carrot fibre was 33.94±0.64 g/100 mL and the packed density was 42.84±1.05 g/100 mL. The density normally depends on the structural characteristics of each material such as the particle shape, size and size distribution. The pineapple core dietary fibre was shown to have a higher density at larger particle size (Prakongpan et al., 2002). The densities of carrot fibre were similar to those of most other fibre products (Table 3.4), except pineapple core fibre, which had relatively low values.

Table 3.2. Dietary fiber contents of some agricultural by-products (%, w/w)

Origin of fiber	IDF	SDF	TDF	References
Carrot	17.64	4.76	22.4	This study
Wheat bran	41.1	2.9	44.0	Grigelmo-Miguel and Martin-Belloso (1997)
Oat bran	20.2	3.6	23.8	Grigelmo-Miguel and Martin-Belloso (1997)
Peach	20.0-23.8	10.7-12.3	30.7-36.1	Grigelmo-Miguel et al. (1999)
Orange	22.8-25.5	11.3-13.0	35.4-36.9	Grigelmo-Miguel and Martin-Belloso (1999)
Onion	9.2-26.6	4.4-6.7	14.9-31.0	Jaime et al. (2002)

Table 3.3. Particle size distribution of carrot fiber residue

Particle size		Distribution (%)
µm	mesh	
125-180	120-80	0.44
180-250	80-60	7.82
250-425	60-35	70.50
425-500	35-32	20.26
500-1000	32-16	0.97

Table 3.4. Density of some agricultural fiber by-products

Origin of fiber	Bulk density (g/mL)	Packed density (g/mL)	References
Carrot	0.34	0.43	This study
Wheat bran	0.39	0.43	Chen et al. (1988)
Oat bran	0.42	0.61	Chen et al. (1988)
Apple	0.46	0.66	Chen et al. (1988)
Peach	0.525-0.627	----	Grigelmo-Miguel et al. (1999)
Orange	0.359-0.382	----	Grigelmo-Miguel and Martin-Belloso (1999)
Pineapple core	0.148	0.171	Prakongpan et al. (2002)

3.3.2.3. Water binding capacity

The water binding capacity of the carrot fibre was 7.64 mL/g on dry matter basis, which was higher than that of other grain fibres and similar to that of fruit fibres (Table 3.5). This high WHC makes carrot fibre not only a functional component for human gastrointestinal health, but also a potential functional ingredient to reduce calories, avoid syneresis and modify the viscosity and texture of formulated foods. The WBC of a fibre is affected by its chemical composition and physical structure. WBC is a measure of the amount of water, which can be bound and trapped by the fibre. So, the differences in the WBC of fibres reflect the differences in their structure that affect its ability to entrap water.

Some other physical properties such as porosity, density and particle size all affect the WBC. Ang (1991) found that the WBC of the powdered cellulose increased to a great extent with fibre length between 35 and 100 µm, when eight fibre lengths (11, 17, 22, 35, 50, 60, 110, and 290 µm) were studied. It was also reported by Cadden (1987) that decreasing the particle size of wheat bran decreased the WBC. However, oat bran and microcrystalline cellulose demonstrated higher WBC with reduced particle size (Cadden, 1987). Therefore, the particle size effect on WBC is fibre specific and is mainly controlled by predominant components. For example, in the case of sugar beet and citrus, a reduction in particle size had a depressing effect on WBC, as sugar beet and citrus are mainly composed of the primary cell wall components such as pectins, which provide the fibre a loose network of polysaccharides and therefore a hydrophilic and elastic property (Auffret et al., 1994). On the other hand, for wheat bran and pea hulls, WBC increased

Table 3.5. Water binding capacity (WBC) and oil binding capacity (OBC) of some agricultural fiber by-products

Origin of fiber	WBC (g/g)	OBC (g/g)	References
Carrot	7.64	1.88	This study
Wheat bran	3.0-6.8	----	Auffret et al. (1994)
Oat bran	2.1	----	Chen et al. (1988)
Pea hulls	4.3-6.2	----	Auffret et al. (1994)
Sugar-beet	19.2-24.2	----	Auffret et al. (1994)
Citrus	8.6-10.9	----	Auffret et al. (1994)
Apple	9.36	----	Chen et al. (1988)
Peach	9.2-12.1	1.02-1.11	Grigelmo-Miguel et al. (1999)
Orange	7.3-10.32	0.86-1.27	Grigelmo-Miguel and Martin-Belloso (1999)
Pineapple core	10.3-12.16	3.77-3.91	Prakongpan et al. (2002)

with a reduction in particle size, which is mainly due to its high amount of secondary cell wall components like crystalline cellulose, imparting the fibre rigidity and poor hygroscopic properties (Eastwood, 1973).

Two possible mechanisms have been proposed about the depressing effect of a reduction in particle size on WBC of certain fibres. Auffret et al. (1994) reported that the effect of grinding on WBC is not only due to the effect of reduction in particle size, but also attributed to the effect of changing the physical structure of the matrix, as grinding caused the collapse of the pores in the matrix structure and changed the spaces available for free water. However, according to Heller et al. (1977), a reduction in particle size results in a significant decline in the estimated hemicellulose content, a constituent largely responsible for hydrophilic properties and therefore water-holding capacity.

The positive effect of grinding on WBC was also explained by Auffret et al. (1994), when the hydration property of pea hulls was studied. They concluded that because of its rigidity, pea hulls resist grinding and the fibre matrix is altered to a lesser extent. The main effect of grinding is to increase the surface area and pore volume, which leads to a higher ability to hold water. However, Cadden (1987) had a different explanation on the effects of particle size reduction on the physical structure change of oat bran. He described that when oat groats were steam-treated and kiln-dried to inactivate the lipase present in the bran layers of the groat, the layers of the plant cell wall were collapsed. Therefore, grinding had no further effect on disrupting the physical structure. However, the matrix structure of wheat bran remained intact after they were separated from the endosperm during roller milling. So, the wheat bran cell wall was sheared upon grinding and the matrix structure collapsed. No matter whether the reduced particle size has a positive or negative effect on the WBC, the smaller the particle size, the higher the initial water adsorption rate, which might be attributed to a better accessibility of the surface capillaries (Auffret et al., 1994).

Another factor that also has some effect on the WBC is pH. Powdered cellulose was observed to retain more water at higher pH and the effect of pH on the WBC was greater in cellulose with longer fibre lengths (Ang, 1991). The ability of the fibre to bind water could be altered when used in different foods, since the presence of other hydrogen-bonding ingredients, such as acids and sodium chloride, tends to interfere with bond sites available between fibres and water (Prokongpan et al., 2002).

3.3.2.4. Swelling capacity

Like WBC, swelling capacity is another hydration property. Water binding capacity represents the hydration ability of fibre by the amount of water held under centrifugal forces, while swelling capacity is the volume increase after the fibre absorbs and holds a certain amount of water under the force of gravity. Dietary fibre interacts with water mainly through two mechanisms, namely, surface tension strength and hydrogen bonding or dipole forms (Labuza, 1968). Therefore, the WBC is the measurement of the water fraction that interacts with fibre molecules and swelling capacity is the measurement of water that represents the water interacting and weakly binding to the fibre as a result of the surface structure through surface tension strength.

The swelling capacity of the carrot fibre residue was 10 mL/g. If expressed as the percentage increase in particle volume upon hydration, the volume expansion of fibre was 400%. The swelling capacity of carrot fibre was similar to that of other fibre products, but extremely lower than that of guar gum (Table 3.6). Fibre with a high swelling capacity is of great importance to food processors from the economical standpoint, as it can increase product yield and the shelf stability for some formulated products. The volume increase of carrot fibre upon pressing was 3.5 mL/g dry carrot fibre. If expressed as a percentage increase, the volume of the particles with swollen cell structure was 140% higher than its original volume.

The swelling capacity of fibre depends on the properties of the individual components and thereafter the physical structure of the fibre matrix, especially the porosity. The fibre acts like a bundle of glass capillaries, which will wet, swell and take up more water by the capillary suction pressure (Chen et al., 1984). Therefore, the more porous is the fibre, the greater is the amount of water taken up. Any factor that affects suction pressure, such as the surface tension (can be defined as the net effect of intrinsic forces between water molecules and the adhesive forces between water molecules and food components), capillary length and capillary diameter will influence the swelling capacity. The surface tension of the fibre strongly depends on the physicochemical properties of the food components. The capillary length and diameter are closely related to the particle size. The swelling capacity of fibre was found to decrease with particle size and was explained by the level of damage to the fibre matrix (Auffret et al., 1994). For some fibres with strand and stick shapes, another possible reason might be the reduced length of particles.

Table 3.6. Swelling capacity of some agricultural fiber by-products

Origin of fiber	Swelling capacity (mL/g)	References
Carrot	10	This study
Wheat bran	5.9-11.9	Auffret et al. (1994)
Pea hulls	7.8-9.9	Auffret et al. (1994)
Sugar-beet	15.9-21.5	Auffret et al. (1994)
Citrus	13.3-15.7	Auffret et al. (1994)
Cauliflower-floret (freeze-dried)	19.4	Femenia et al. (1999)
Artichoke flour	10.98	Lopez et al. (1996)
Guar gum	84.02	Lopez et al. (1996)

3.3.2.5. Oil binding capacity

The oil binding capacity of carrot fibre was 1.88 ± 0.09 g oil /g fibre, which was higher than that of orange and peach fibres and lower than that of pineapple core fibre (Table 3.5). As all fibres are hydrophilic, the amount of oil retained by fibres is much lower than their water retention. The OBC makes dietary fibre appropriate for stabilization of foods with a high percentage of fat and emulsions.

The oil binding capacity of fibre is related to the hydrophilic nature of the surface and density or thickness of the particles. The particles with a greater surface area theoretically present a greater capacity to adsorb and bind lipid components (Lopez, 1996). Sosulski and Cadden (1982) found that lignin-rich samples had more oil binding capacity when studying different sources of DF.

3.3.2.6. pH

The pH of the 10% carrot fibre solution was 5.81 ± 0.02. The pH varies with the type of fruit and vegetable. The pH of carrot fibre was higher than that of orange and peach fibres at the same concentration and similar to that of pineapple core fibre at approximately the same concentration (Table 3.7). Even within the same product, the pH varies with variety, region and cultivation (Grigelmo-Miguel and Martin-Belloso, 1999).

3.3.2.7. Energy

As a result of the low proportion of high-energy components, the gross energy of the carrot fibre was only 3,814.43 ± 3.35 cal/g, similar to that of orange and peach fibres, which was 3,494-3,723 and 3,519-3,735 cal/g, respectively (Grigelmo-Miguel and

Table 3.7. pH of some fruit fibers

Origin of fiber	pH	References
Carrot	5.81	This study
Peach	3.85-3.93[a]	Grigelmo-Miguel et al. (1999)
Orange	3.63-3.86[a]	Grigelmo-Miguel and Martin-Belloso (1999)
Pineapple core	6.14-6.35[b]	Prakongpan et al. (2002)

[a] For a 10% solution

[b] For 2 g sample in 18 mL solution

Martin-Belloso, 1999; Grigelmo-Miguel et al., 1999). Therefore, carrot fibre can be used as a low-calorie bulking agent in reduced calorie foods.

3.3.2.8. Color

Compared with the feed material, the carrot fibre had a light yellowish orange color, which is caused by the unparallel removal of carotene and lutein. The color parameters of the carrot particles before and after extraction are listed in Table 3.8. Compared with the feed carrot material, the redness ‘a’ dropped greatly and the yellowness ‘b’ dropped only slightly. This indicated that more carotenes were removed than lutein in the extraction process. The brightness ‘L’ increased because of the reduction in red and yellow color. The hue value of the extraction residue decreased dramatically from 1.24 to 0.32. The decrease of the hue value can either be caused by a reduction in ‘a’ or an increase in ‘b’. Since both ‘a’ and ‘b’ values decreased, the only explanation is the greater decrease of ‘a’ compared to ‘b’. Chroma dropped as both ‘a’ and ‘b’ decreased. The ΔE value is an indication of the overall color change after extraction. The bigger the ΔE value, the greater the extraction effect on the removal of the pigments. The very light color of the carrot fibre may slightly affect the color of the food products it may be incorporated into. However, this light color of carrot fibre may be easily masked by other food ingredients that have strong color. Alternatively, extraction may be carried out for a longer period of time to ensure that all pigments are removed.

Table 3.8. Color parameters of carrot feed material and residue

Color parameter	Feed material	Extraction residue
'L'	49.58	65.36
'a"	31.79	7.35
'b"	25.57	22.62
Hue	1.24	0.32
Chroma	40.80	23.78
ΔE	29.24	

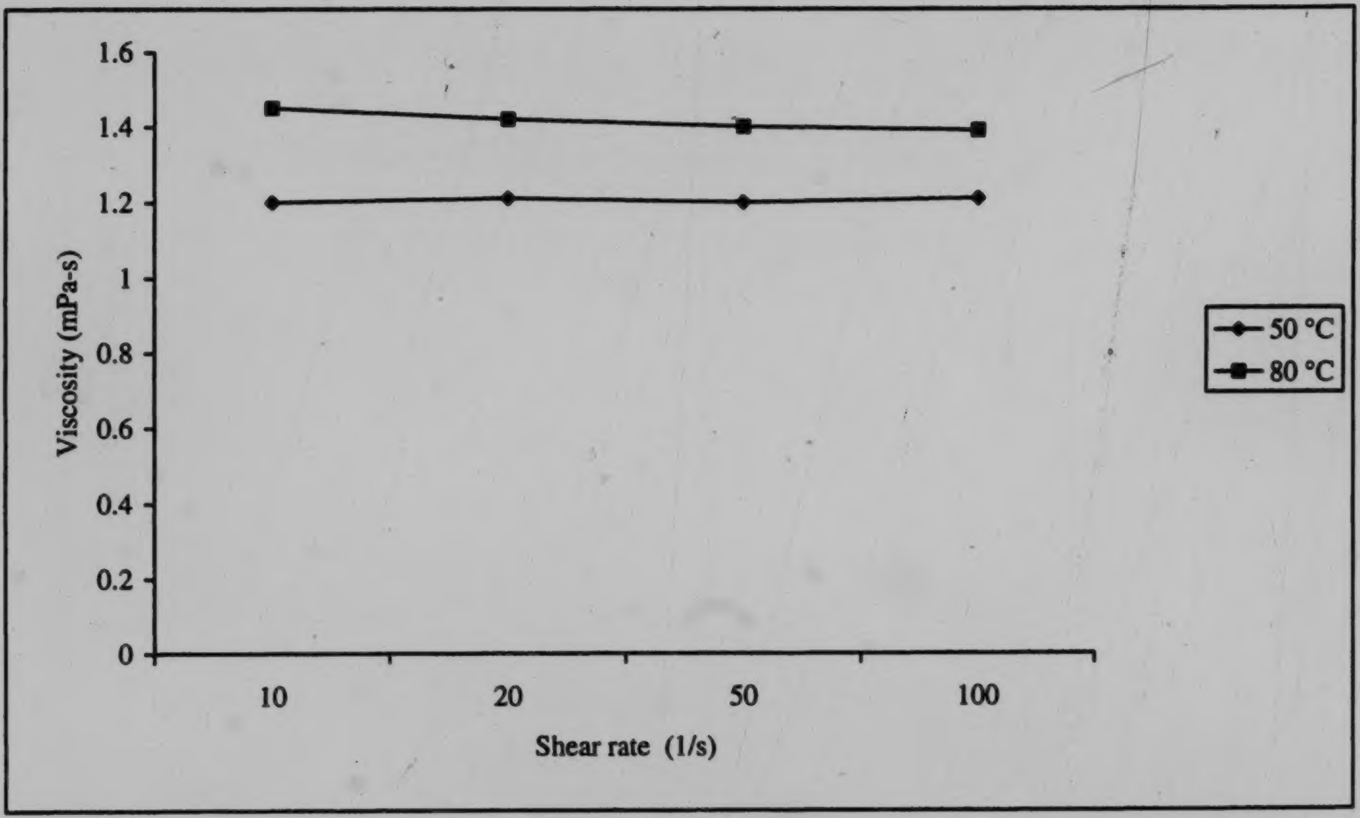

Figure 3.1. Viscosity of carrot fiber solutions (5% w/w) prepared at 50 and 80 °C (viscosity measured at 20 °C)

Table 3.9. Viscosity of some fibers and gum

Origin of fiber	Viscosity (mPa.s)	References
Artichoke	0.99-1.19[a]	Lopez et al. (1996)
Guar gum	210.56[b]	Lopez et al. (1996)
Pineapple core dietary fiber	14.5[c]	Prakongpan et al. (2002)
Pineapple core cellulose	17.5[d]	Prakongpan et al. (2002)

[a] 2% suspension at 37 °C and 1.83 s^{-1} shear rate

[b] 2% solution at 37 °C and 73.42 s^{-1} shear rate

[c] 2% suspension at room temperature and 122.36 s^{-1} shear rate

[d] 4% suspension at room temperature and 122.36 s^{-1} shear rate

3.3.2.9. Viscosity

The supernatants of the fibre solutions were measured at 20 °C to test the viscosity. The viscosities of the carrot supernatants were only 1.2 and 1.4 mPa-s, after being cooked at 50 and 80 °C, respectively (Fig. 3.1). This was not surprising since the soluble fibre content of the carrot fibre was only 4.76%. In some other studies, the viscosity of fibre suspensions were measured (Table 3.9). The viscosity of a suspension depends on molecular shape, size and charges (Whistler and Daniel, 1990). Generally, long linear molecules have greater resistance to shear than short or branched molecules and larger particles give higher viscosity. Powdered cellulose solutions (1, 2, and 3% w/v) showed very low viscosity with fibre length of 35 and 60 µm. However, the viscosity of the suspensions containing a 110 µm cellulose powder increased 5 times at 1%, 10 times at 2%, and 27 times at 3% (Ang, 1991). Prakongpan et al. (2002) also found that cellulose fibres smaller than 110 µm appeared to have no thickening properties. Svanberg et al. (1995) reported that the viscosity of carrot soluble fibre was concentration dependent. They found that the maximum viscosities of the carrot soluble fibre were 200, 1700 and 7000 mPa-s at the concentration of 2.5, 4 and 5%, respectively, showing an exponential increase with concentration. The viscosity is pH dependent especially for polysaccharides with charged side groups. When the effects of three pH values (2.4, 2.9, and 3.6) on the viscosity of pectin were studied, Svanberg et al. (1995) found that pectin showed a maximum viscosity at pH 2.9.

3.3.2.10. Gel formation

When the carrot fibre was dispersed in water, there was no gel formation, even after they were cooked at elevated temperatures (50 and 80 °C) followed by cooling. However, the fibre became swollen very quickly. If the concentration was high, for example 20% (w/w), a turbid paste was formed and if the concentration was low, for example 5% (w/w), a turbid suspension was formed. The fibre particles settled out of the suspension if it was allowed to stand without agitation.

3.3.3. Antioxidant activity of oil extract and fibre residue

3.3.3.1. Antioxidant activity of oil extract

The total weight of the carotenoid crystals from ten extractions was 2.02 g and the total oil saturated with carotenoids was 248.99 g. The α-carotene and β-carotene concentrations in the crystals were 54.7 and 159.71 mg/g, and those in the oil were 7.44 and 10.26 mg/mL, respectively. The antioxidant activity of the carotenoid crystals and the oil saturated with carotenoids was demonstrated in Figure 3.2 together with that of the fibre residue and BHT. Butylated hydroxytoluene was chosen for this comparison, since it is a potent synthetic antioxidant used extensively in the food industry. The concentration of BHT and crystallized carotenoids were 0.5 g/L and that of the oil saturated with carotenoids was 0.2 L/L. The antioxidant activity of the carotenoid crystal (34.96) was approximately one-third of that of BHT. However, the antioxidant activity of the oil saturated with carotenoids (88.61) was only slightly lower than that of BHT (98.6). The difference in the antioxidant activity of these samples may be due to the differences in the actual carotenoid contents of these products.

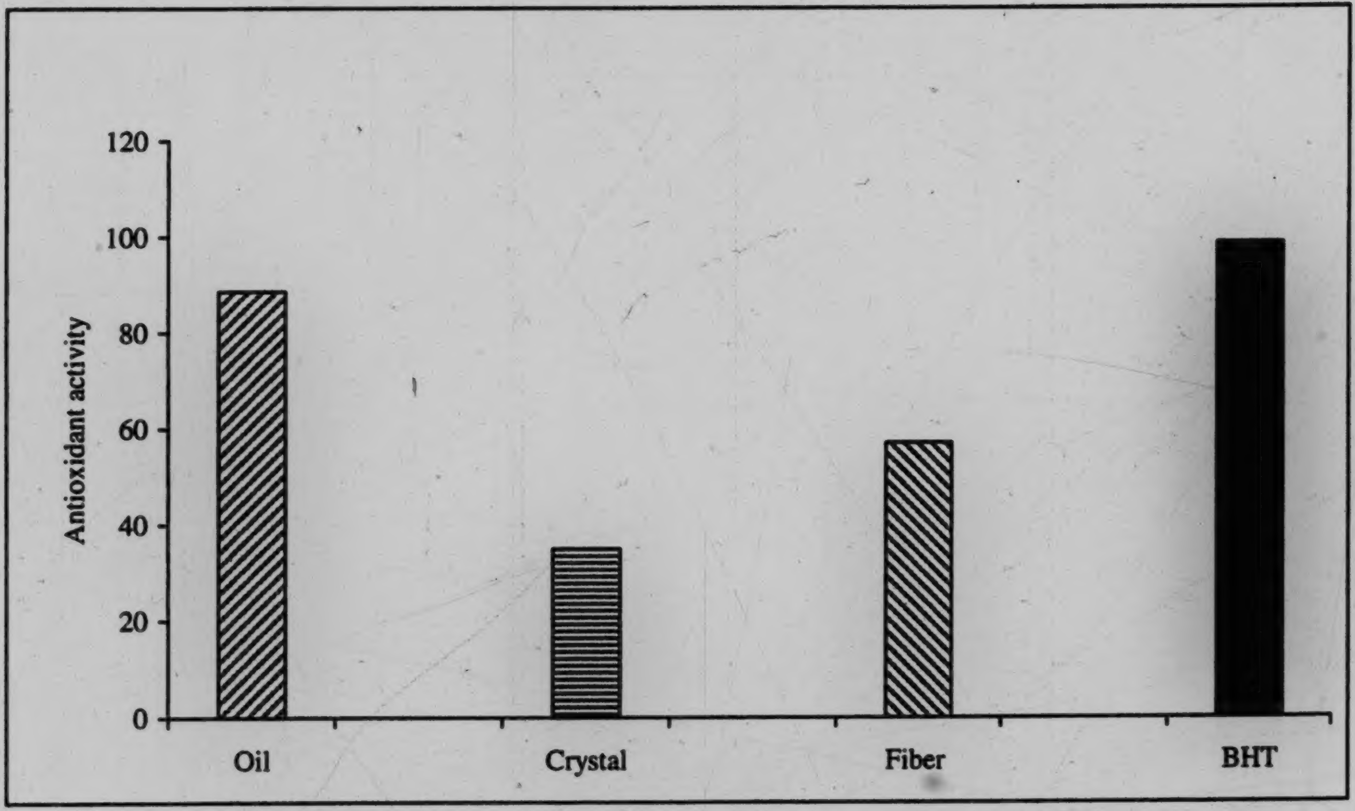

Figure 3.2. Antioxidant activity of extracts and fiber compared with that of BHT

3.3.3.2. Antioxidant activity of fibre residue

Antioxidant activity of fibre is a health-promoting property derived from the bioactive compounds associated with dietary fibre. The absolute amount of extract from the three samples was 0.0045± 0.0022 g, which was 0.22% of the carrot fibre. Therefore, the concentration of the antioxidant activity assay mixture was 1.875 g/L, which was 3.75 times higher than that of BHT. As shown in Figure 3.2, the carrot fibre had a promising antioxidant activity (57) compared with BHT and other carrot products, despite the fact that this result was achieved at a higher concentration. In addition to the properties derived from ordinary dietary fibres, prevention of lipid oxidation in food products can be expected from the presence of residual antioxidant carotenoids. On the other hand, the potential of the combined actions of carotenoids and other antioxidants like tocols (tocopherols and tocotrienols) are very promising in nutrition and health.

The antioxidant activities of plant dietary fibres were also studied by Larrauri et al. (1996 and 1997), Saura-Calixto (1998) and Jimenez-Escrig et al. (2001) with pineapple core, red grape peel and guava fruits, respectively. In those three studies, the antioxidant activities were all attributed to the associated polyphenolic compounds. Larrauri et al. (1996) found that mango peel dietary fibre had 74.7% antioxidant activity at 0.05% fiber extract concentration. Larrauri et al. (1997) showed that commercial lemon and apple fibres had no antioxidant activity and commercial orange fibre exhibited low (34.6%) antioxidant activity compared to that of the pineapple shell fibre (86.7%). Saura-Calixto (1998) found that 0.7 g polyphenol extract solution (containing 14 mg extractable polyphenols) can reduce the oxidation of linoleic acid by 50%.

3.3.4. Effect of extraction parameters on the color of residue

3.3.4.1. Effect of temperature, pressure and canola oil concentration

The color parameters of the carrot residue obtained at different combinations of temperature, pressure and canola oil concentration were listed in Table 3.10. The main and interaction effects of the three factors were shown in Figure 3.3 and Table 3.11. As expected, the intensities of redness 'a' and yellowness 'b' of the carrot particles decreased after extraction at all conditions studied, while the brightness 'L' increased after extraction. In general, the higher yield of extracted pigments corresponded to a greater reduction in the redness and yellowness. The brightness increase may be due to the loss of redness and yellowness and therefore the particles become whiter in color. The drop in redness 'a' increased with temperature and oil concentration, since temperature and oil concentration had a negative linear effect on redness as shown in Figure 3.3b. However, the drop in yellowness 'b' decreased with pressure and oil concentration, because they had a positive quadratic effect on yellowness (Fig. 3.3c). This suggests that higher temperature and oil concentration are more favorable for the removal of red tones and higher pressure and oil concentration are not favourable for the extraction of yellow tones. This conclusion was supported by the previous analysis that: 1) high α- and β-carotene yield can be obtained at high temperature and oil concentration, as α- and β-carotene are the main carotenoids responsible for the red tones, and 2) the oil concentration had a negative quadratic effect on lutein extraction at high concentration, which means that less lutein was extracted at high oil concentration, therefore imparting the residue more yellow tones. Figure 3.3a shows that the brightness of the residue

Table 3.10. Color parameters of extraction residue and feed material

Color peameters			'L'	'a'	'b'	Hue	Chroma	ΔE
Feed material			57.27	31.45	37.1	0.85	48.64	----
0% canola oil	40 °C	27.6 MPa	68.22	15.77	28.36	0.56	32.45	21.03
		41.3 MPa	68.24	16.86	27.18	0.62	31.98	20.78
		55.1 MPa	68.9	14.95	26.79	0.56	30.68	22.67
	55 °C	27.6 MPa	69.87	12.74	27.85	0.46	30.63	22.56
		41.3 MPa	69.58	13.66	27.52	0.50	30.72	21.63
		55.1 MPa	69.2	13.97	28.92	0.48	32.12	21.16
	70 °C	27.6 MPa	69.85	10.97	29.99	0.37	31.93	24.04
		41.3 MPa	71.23	9.97	30.03	0.33	31.64	25.62
		55.1 MPa	70.42	10.56	30.78	0.34	32.54	24.68
2.5% canola oil	40 °C	27.6 MPa	72.41	9.34	24.35	0.38	26.08	26.80
		41.3 MPa	72.33	9.66	23.86	0.40	25.74	26.49
		55.1 MPa	68.88	12.62	30.07	0.42	32.61	22.12
	55 °C	27.6 MPa	73.29	7.93	24.19	0.33	25.46	28.46
		41.3 MPa	72.31	8.94	24.85	0.36	26.41	27.07
		55.1 MPa	68.85	11.54	29.88	0.39	32.03	23.03
	70 °C	27.6 MPa	67.25	9.51	33.23	0.29	34.56	24.10
		41.3 MPa	73.61	6.65	24.76	0.27	25.64	29.70
		55.1 MPa	73.06	7.27	26.56	0.27	27.54	28.88
5% canola oil	40 °C	27.6 MPa	67.83	10.43	31.87	0.33	33.53	23.52
		41.3 MPa	70.76	9.23	26.97	0.34	28.51	25.99
		55.1 MPa	64.05	13.59	37.57	0.36	39.95	19.10
	55 °C	27.6 MPa	69.27	9.62	31.51	0.31	32.95	24.91
		41.3 MPa	70.92	8.86	28.47	0.31	29.82	26.39
		55.1 MPa	68.85	10.36	31.89	0.32	33.53	24.06
	70 °C	27.6 MPa	69.95	8.07	30.41	0.27	31.46	26.60
		41.3 MPa	72.46	6.93	27.53	0.25	28.39	28.84
		55.1 MPa	67.55	9.59	32.75	0.29	34.13	24.16

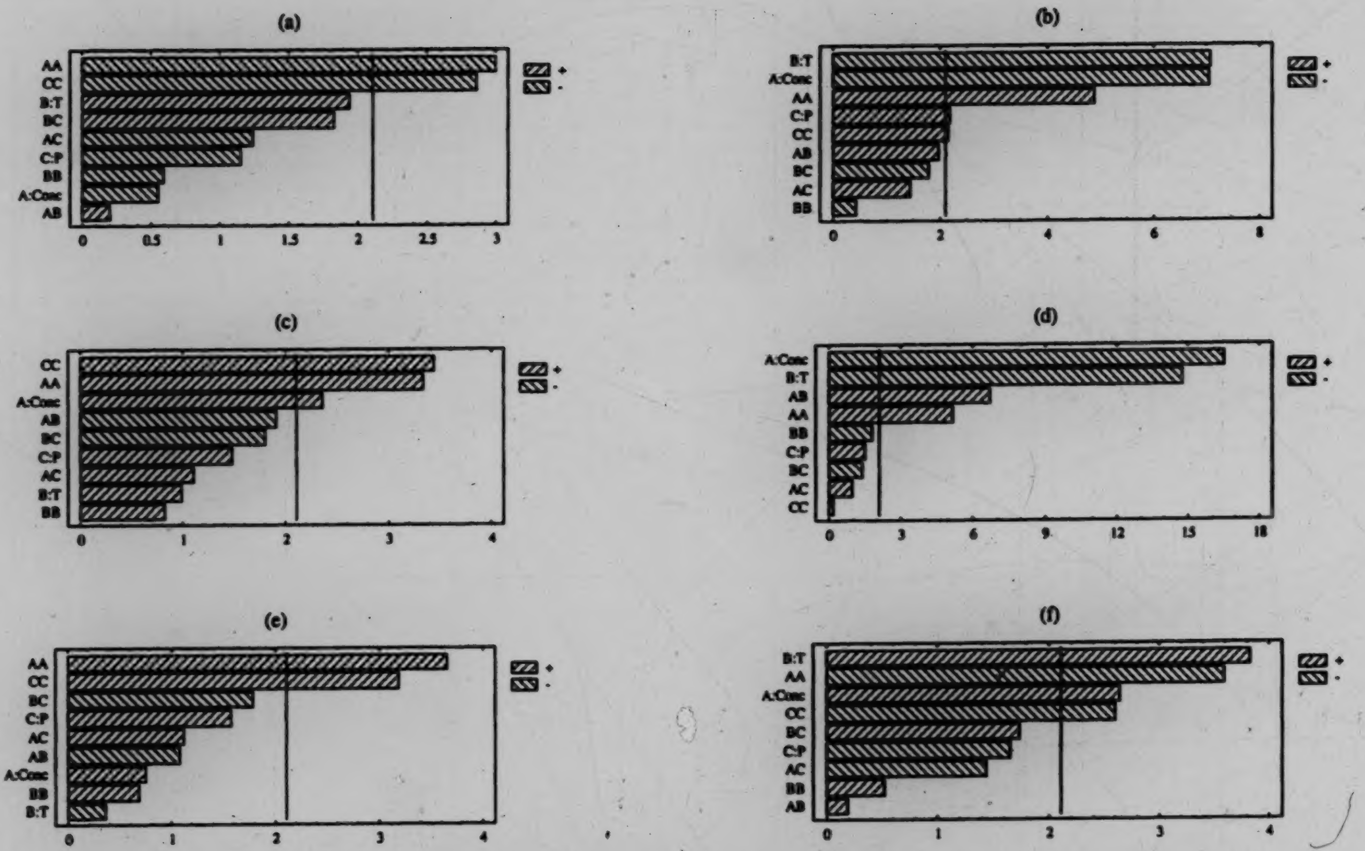

Figure 3.3 Standrd Pareto chart for color parameters of carrot extract residue, (a) brightness 'L', (b), redness 'a', (c), yellowness 'b', (d) hue, (e) chroma and (f) ΔE (A, B and C represent the concentration of canola oil, temperature and pressure, respectively, and AA, BB, CC, AB, AC and BC represent the interaction among them)

Table 3.11. The p value of analysis of variance for color parameters

Source[1]	'L'	'a'	'b'	Hue	Chroma	ΔE
A: Conc.	0.8971	0.0000	0.0630	0.0000	0.6107	0.0112
B: T	0.0377	0.0000	0.4768	0.0000	0.6194	0.0010
C: P	0.4847	0.0882	0.2556	0.2849	0.2160	0.2371
AA	0.0141	0.0003	0.0070	0.0002	0.0036	0.0043
AB	0.5165	0.1432	0.0718	0.0000	0.2565	0.5613
AC	0.4967	0.3013	0.4525	0.5902	0.4281	0.3727
BB	0.7274	0.6237	0.5112	0.0722	0.5896	0.4819
BC	0.0435	0.0868	0.0856	0.1193	0.0880	0.0586
CC	0.0186	0.0699	0.0057	0.7123	0.0093	0.0313

[1] A, B and C represent the concentration of canola oil addition, temperature and pressure of extraction, respectively and AA, AB, AC, BB, BC and CC represent the interactions among them.

dropped due to quadratic effect of pressure and oil concentration. This might be contributed by the remaining lutein at these conditions.

Hue and chroma values calculated based on the 'a' and 'b' values both decreased compared to the feed material. The decrease in hue value means that the decrease in redness was greater than that in yellowness, while the decrease in chroma value once again indicated the overall decrease in redness and yellowness. Following the trend in redness 'a' with an increase in temperature and oil concentration, the hue value decreased with temperature and oil concentration (Fig. 3.3d). It suggested that carotenes are the determinant factor for the hue values. This further demonstrated the preferential removal of redness at higher temperatures and oil concentrations. Chroma value increased due to quadratic effects of oil concentration and pressure (Fig. 3.3e). It once again demonstrated that more lutein was retained at high pressure and oil concentration. It also suggested that lutein is the determinant of chroma value. Figure 3.3f shows that the overall color change in carrot particles before and after extraction increased with temperature, decreased with pressure and increased with oil concentration at low concentration levels and then decreased with oil concentration at high concentration levels (based on the previous discussion in Chapter 2, Section 2.3.2.2).

3.3.4.2. Particle size

All color parameters (p values were 0.0013, 0.0065, 0.0265, and 0.0058 for 'L', 'a', hue and ΔE, respectively) except yellowness 'b' and chroma were affected by particle size. As shown in Figure 3.4, the smaller the particle size, the more carotenoids were

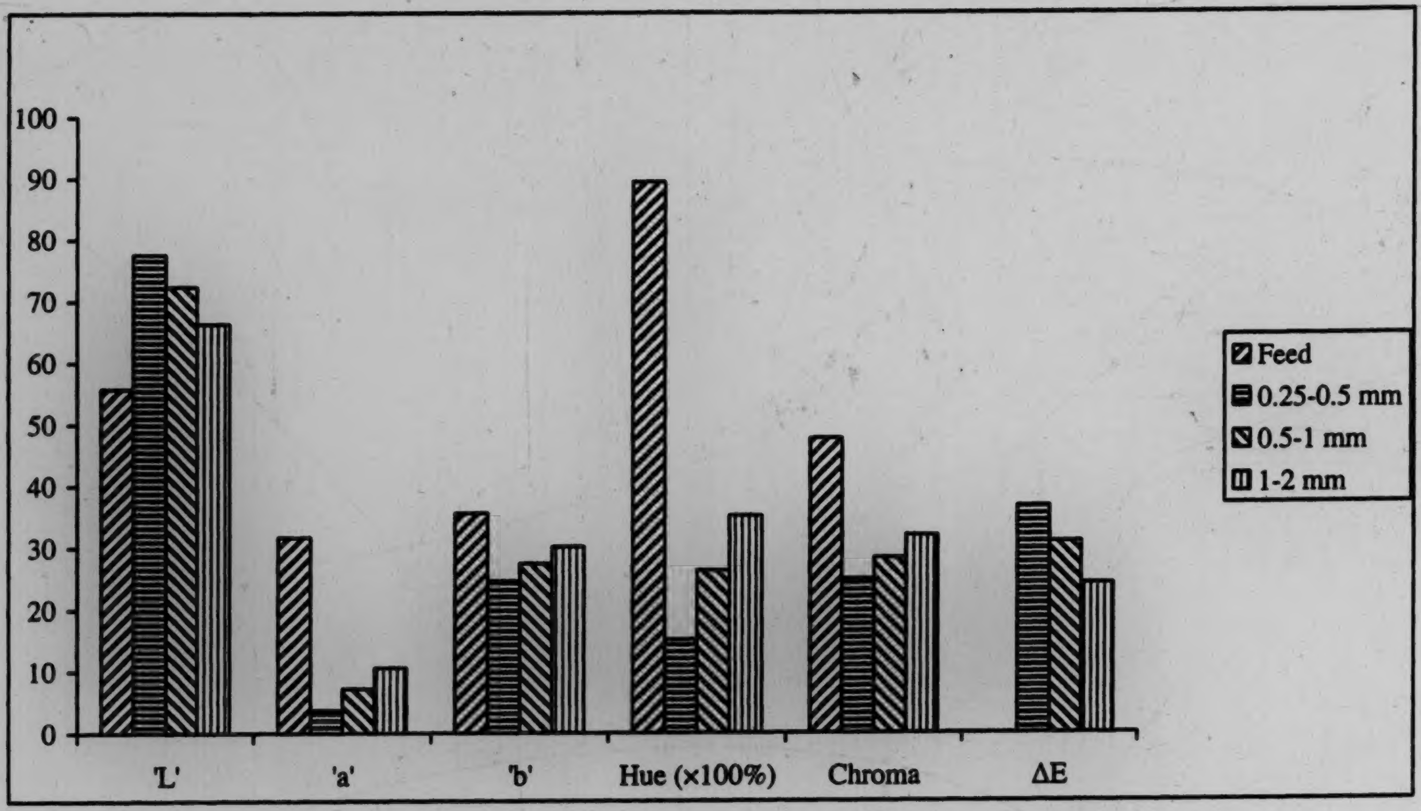

Figure 3.4. Color parameters of feed material and extraction residue with different particle sizes

extracted, especially red color components. The more the pigments were removed, the brighter were the particles.

3.3.4.3. Moisture of feed material

The statistical analysis showed that the moisture content of the feed material had significant effects on redness 'a' (p=0.0001), and therefore on brightness 'L', hue, and ΔE (p values were 0.0017, 0.0013 and 0.0009, respectively). If only the three wet moisture levels were considered, they had a significant effect on redness and hue (p values were 0.0079 and 0.0219, respectively). The reduction in redness increased with decreasing moisture and the dry material gave the greatest reduction in redness (Fig. 3.5). It indicated that the dry sample was the optimum starting material for the extraction of red color compounds. As a consequence of the moisture effect on the redness and yellowness, the brightness was not significantly different among wet samples, but were different between dry and wet samples. Hue values were different among wet samples as well as between dry and wet samples. The trend of hue value was similar to that of redness. The overall color change ΔE was significantly different between dry and wet samples (p=0.0009), but not among wet samples.

3.3.4.4. CO_2 flow rate

Although the color parameters of residue were different from those of feed, the flow rate had no significant effect on all the parameters studied as shown in Figure 3.6.

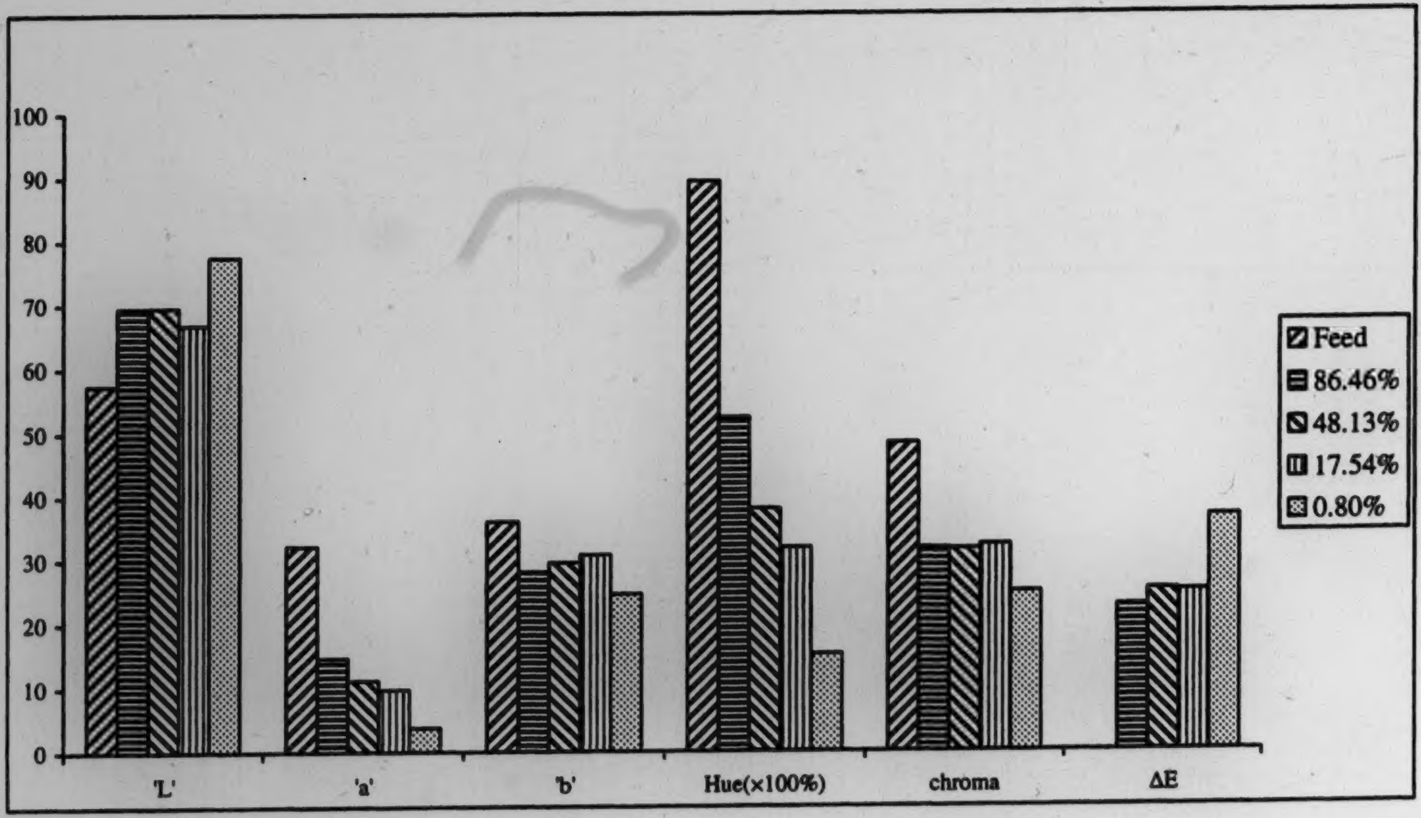

Figure 3.5. Color parameters of feed material and residue after extraction at different moisture levels

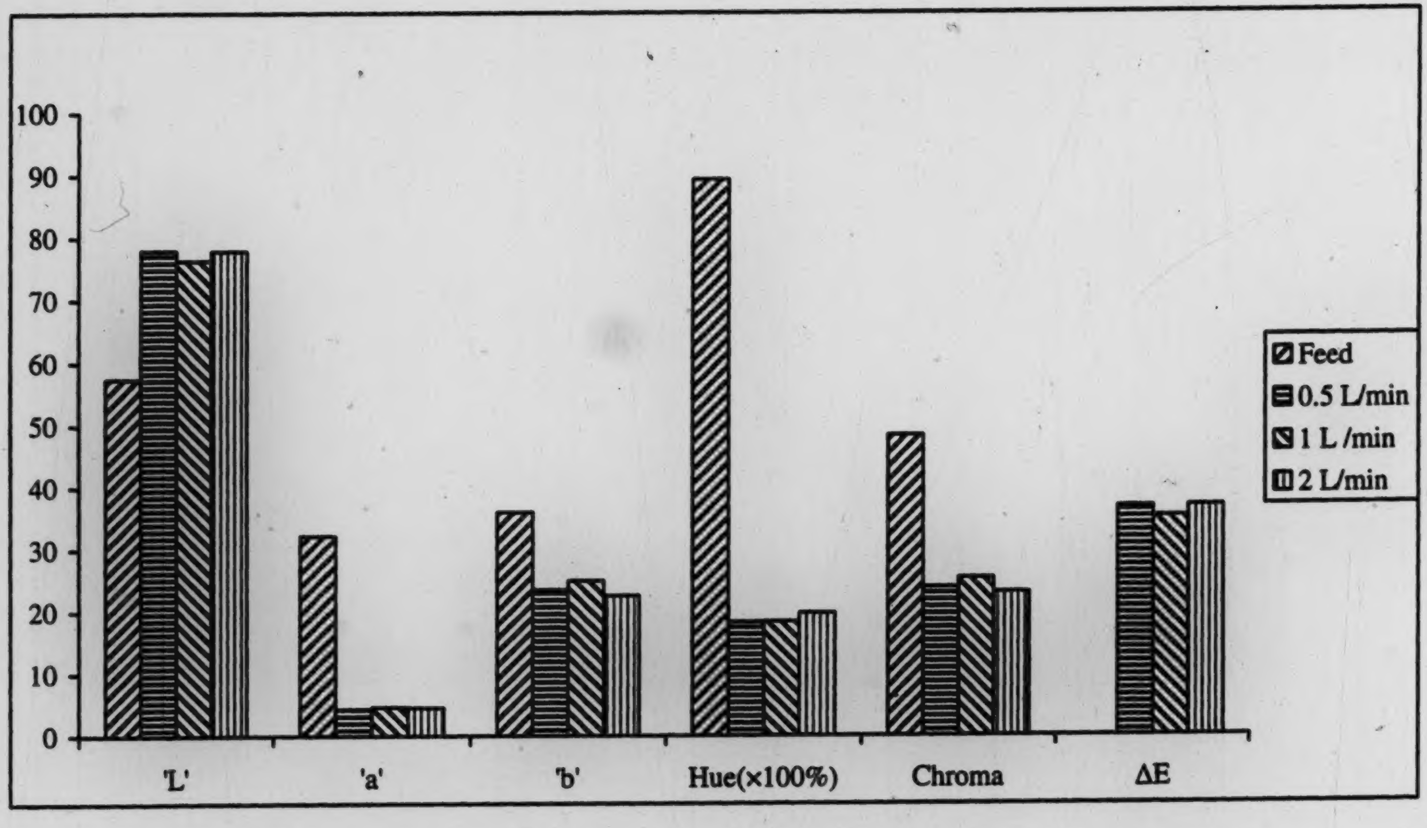

Figure 3.6. Color parameters of feed material and residue after extraction at different flow rates

3.4. CONCLUSIONS

The carrot fibre residue from the SC-CO_2 extraction had a reasonable amount of dietary fibre and ratio between insoluble and soluble portions, which was similar to those of oat bran. The carrot fibre had a relatively high WBC, OBC and swelling capacity, which makes carrot fibre a potential bulking agent and a functional ingredient in various food applications. The light color and low energy of carrot fibre are two desirable characteristics in low-calorie foods. The antioxidant activity of carrot fibre imparts it another important physiological effect. Therefore, the carrot fibre obtained from SC-CO_2 extraction is a potential functional component from both nutrition and food applications point of view. Both the SC-CO_2 extracted carotenoid crystals and the carotenoid-saturated canola oil had promising antioxidant activity. Color analysis of carrot particles revealed that carotenes were the carotenoids responsible for the red tones and lutein was the one responsible for the yellow tones of the carrot. Carotenes were removed to a greater extent than lutein, especially at higher temperature and canola oil concentration. Therefore, the extraction yield of carotenes and lutein can be adjusted by changing the extraction conditions. It was demonstrated that SC-CO_2 extraction of carrots can produce carotenoid extract and fibre residue products with antioxidant activity.

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4. CONCLUSIONS AND RECOMMENDATIONS

4.1. CONCLUSIONS

Carotenoids and dietary fibre are two major nutraceutical components in carrots. These two products can be obtained by both the traditional solvent extraction (TSE) and supercritical carbon dioxide (SC-CO_2) extraction. However, the TSE, which generally requires the application of heat to remove organic solvent from the products, can result in the degradation of a considerable amount of carotenoids, whereas the extract products obtained from SC-CO_2 extraction have no solvent residue.

The solubility of carotenoids in SC-CO_2 is extremely low. Therefore, a suitable co-solvent is needed to improve the extraction efficiency. Addition of an organic co-solvent, which later needs to be removed from the extract, has been reported in previous studies. Using vegetable oil as a co-solvent in SC-CO_2 extraction has not been reported previously. Employing canola oil as a co-solvent, SC-CO_2 extraction was proven to be an efficient technique to recover both natural pigments (carotenoids) and dietary fibre from carrots. Such an approach shows great promise for commercial application.

4.1.1. Effect of extraction parameters on SC-CO_2 extraction of carotenoids from carrot

Compared with TSE, SC-CO_2 extraction without canola oil addition can recover approximately the same amount of crude carrot oil and half the amount of carotenes. However, a significant amount of lutein was recovered with SC-CO_2 extraction, which was not possible with TSE. When canola oil was added as a co-solvent, the α- and β-carotene yields were improved more than twice and lutein yield was more than four times compared to those obtained with SC-CO_2 extraction alone. The crude carrot oil was not quantified in the oil sample collected from SC-CO_2 extraction with canola oil addition, since it was not possible to separate it from canola oil. Temperature and pressure both had significant positive effects on the carotene yields but not no lutein yield. Compared with pressure, temperature had a more significant effect and the effect of temperature was especially pronounced at high canola oil concentration.

Particle size had a negative effect on carotenoid yields because of the longer diffusion distance of carotenoids and less contact surface between carotenoids and the solvent mixture associated with large particle size. Water was co-extracted when wet carrot samples were used as feed material and the amount of water co-extracted increased with the moisture content of feed material. The α- and β-carotene yields decreased with moisture while the lutein yield increased. This is caused by the slight difference in polarity, as water can act as a co-solvent for the extraction of relatively polar lutein, whereas the presence of moisture is not favourable for the relatively non-polar carotenes. Although a larger amount of oil was obtained at high CO_2 flow rate, the loading of CO_2 was higher at lower flow rates and approached the solubility limit (28.2 mg/ g CO_2) at 0.5 L/min. Higher carotenoid yields were achieved after 4 h of extraction at higher flow rate, while more carotenoids were solubilized in SC-CO_2 at lower flow rate. β-Carotene and lutein reached their solubility equilibrium at flow rates of 0.5 and 1 L/min. The solubility of the total carotenoids was 5.96 μg/g CO_2, and that for α-carotene, β-carotene, and lutein were 3.03, 2.65, and 0.128 μg/g CO_2, respectively.

In the region of the experimental parameters studied, highest carotenoid yields were obtained from the smallest (0.25-0.5 mm) dry carrot particles at highest CO_2 flow rate (2 L/min), with highest temperature (70 °C), pressure (55.1 MPa) and canola oil concentration (5%, w/w).

4.1.2. Characterization of the extract and fibre residue from SC-CO_2 extraction

Three solvent-free products were generated as a result of the SC-CO_2 extraction, namely, carotenoid crystals, carotenoid-saturated canola oil and carrot fibre.

In the extract, the precipitated carotenoid crystals were separated mechanically from the oil part. The separated carotenoid crystals had bright orange and the oil had dark orange color. The carotenoid crystals can be used directly as a "natural" colorant in many fields such as food, pharmaceutical and cosmetic industries, which have high requirements of such ingredients. The carotenoid-saturated canola oil, on the other hand, can be viewed as a carotenoid-enriched edible oil. The SC-CO_2 extraction process can also be viewed as an enrichment process. The carotenoids, mainly α- and β-carotene, not only contribute a desirable color to these products, but also a high antioxidant activity. The antioxidant activity of the carotenoid crystals was 34.96 at 0.5 g crystal/L solution and that of carotenoid-saturated oil was 88.61 at 0.2 L oil/L solution. Due to their function as antioxidants, carotenoids are of great importance not only in the nutritional aspects, but also for various food applications. β-Carotene has been shown to protect lipids from free radical auto-oxidation reactions by reacting with peroxyl radicals and therefore inhibiting the oxidation chain reaction (Palozza and Krinsky, 1991; Britton, 1995). β-Carotene may also inhibit photo-oxidation in vegetable oils by quenching singlet oxygen and filtering light (Mathews-Roth, 1981; Fakourelis et al., 1987).

The carrot fibre contained 22.4% total dietary fibre, with 17.64% insoluble and 4.76% soluble fibre, which was similar to those of oat bran. The high water binding capacity (7.64 g/g) and swelling capacity (10 mL/g) make carrot fibre not only a functional component for human gastrointestinal health, but also a potential functional ingredient in formulated foods. Fibre, with its ability to bind water, can reduce calories, avoid syneresis and modify the viscosity and texture of food products. In addition, it can increase product yield and the shelf stability of some formulated products. The reasonable oil binding capacity (1.88 g/g) of carrot fibre makes it a potential stabilizer in high-fat foods. The low energy (3,814 cal/g) of carrot fibre makes it a low-calorie bulking agent for low-calorie foods. The antioxidant activity of carrot fibre, which is derived from the residual carotenoids, makes the carrot fibre more attractive not only from the nutritional but also from the food applications point of view. The very light color of the carrot fibre makes the application of this fibre in various formulated food products feasible.

Color analysis of carrot particles revealed that carotenes were the components responsible for the red tones and lutein was responsible for the yellow tones of carrots. The effect of extraction parameters on the color was closely related to their effect on carotenoid extraction yields. The drop in redness 'a' increased with temperature and oil concentration, since more carotenes were removed at higher temperature and canola oil concentration. However, the drop in yellowness 'b' decreased with pressure and oil concentration, since less lutein was removed at higher pressure and oil concentration. All the changes in the other color parameters studied (hue, chroma and ΔE) were determined by the relative changes in redness 'a' and yellowness 'b'. The effects of particle size, moisture content of feed material and CO_2 flow rate on the color of fibre residue all corresponded to the relative extent of removal of carotenes and lutein. Therefore, the color of the fibre residue, which is determined by the extent to which the carotenoids are removed, can be adjusted by changing the extraction conditions.

4.2. RECOMMENDATIONS

Since the best conditions for the extraction of carotenoids from carrots using SC-CO_2 determined in the current study were all the extreme conditions within the study domain, more experiments are needed to explore the true optimum conditions. In the case of temperature, as it has two opposite effects on the carotenoid recoveries, the optimum temperature, which can result in a compromise between maximizing carotenoid solubilization and minimizing thermal degradation should be found. In terms of the particle size of the carrot sample, the smallest particle size in the experiment region studied gave the best yield; however, the true optimum particle size can be further investigated based on the fact that smaller particle size can enhance carotenoids yield but would be more prone to channeling effect.

The α- and β-carotene contents of the crystals were only 54.7 and 159.71 mg/g, respectively, and the total dietary fibre only accounted for 22.4% of the weight of the total fibre residue. The composition of the remainder of the crystal and fibre can be further investigated.

The carotenoids in the canola oil are expected to protect against lipid oxidation. Therefore, a storage study comparing the stability of the carotenoid-saturated canola oil and regular canola oil is recommended. In addition, in order to achieve a complete recovery of carotenoids and a lighter color carrot fibre, a longer extraction time and mixing of the sample during extraction should be investigated.

As cellulose and pectin are the two major fibre components that constitute the cell structure of the carrot, addition of cellulase and pectinase enzymes can be investigated to breakdown the cell structure and expose more carotenoids to the solvent mixture, therefore improve the extraction efficiency.

To avoid the accumulation of canola oil in the extraction vessel, the extraction unit can be modified by adding another extraction vessel. The first vessel can be filled with canola oil such that, when the SC-CO_2 passes through the first vessel, it becomes saturated with canola oil. Then, the canola oil saturated SC-CO_2 can flow through the second vessel filled with the carrot sample to extract the carotenoids.

This thesis research demonstrated the potential of recovering the high-value components, carotenoids and dietary fibre, from carrots. Further studies with carrot processing by-product generated in the farm and food processing plants as raw material is strongly recommended.

4.3. REFERENCES

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